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(54) Interleukin-18-receptor proteins

(57) Disclosed are a polypeptide (including that in soluble form) as receptor for a novel cytokine, i.e., interleukin-18, a DNA encoding the polypeptide, and the uses of the polypeptide including pharmaceutical and neutralizer to interleukin-18. Pharmaceuticals with the polypeptide is useful to treat and prevent autoimmune and allergic disease because it suppresses and regulates excessive immunoreaction.

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Description

Background of the Invention

1. Field of the Invention

This invention relates to a novel receptor protein which recognizes a cytokine, more particularly, to a novel polypeptide which recognizes interleukin-18 (hereinafter abbreviated as "IL-18").

2. Description of the Prior Art

IL-18 is a type of cytokine or substance which mediates signal transduction in immune system. As seen in Japanese Patent Kokai Nos.27,189/96 and 193,098/96 and Haruki Okamura et al., *Nature*, Vol.378, No.6,552, pp.88-91 (1995), IL-18 was provisionally designated as "interferon-gamma inducing factor" immediately after its discovery: This designation was changed later into "IL-18" in accordance with the proposal in Shimpei Ushio et al., *The Journal of Immunology*, Vol.156, pp.4,274-4,279 (1996). IL-18 in mature form consists of 157 amino acids and possesses properties of inducing in immunocompetent cells the production of interferon-gamma (hereinafter abbreviated as "IFN-γ") which is known as useful biologically-active protein, as well as of inducing and enhancing the generation and cytotoxicity of killer cells. Energetic studies are now in progress to develop and realize various uses of IL-18 in pharmaceuticals such as antiviral, antimicrobial, antitumor and anti-immunopathic agents which have been in great expectation because of these properties of IL-18.

As described above, in nature, cytokines including IL-18 are produced and secreted as substances responsible for signal transduction in immune system. Therefore, excessive amounts of cytokines may disturb the equilibria in immune system when they are produced or administered in the body of mammals. The surface of usual mammalian cells may bear certain sites or "receptors" which are responsible for recognition of cytokines: Secreted cytokines transduce no signal in cells till they are bound to the receptors. In normal immune system, there would be definite equilibria between respective cytokines and their receptors. Thus, in this field, with the purpose of developing and realizing IL-18 as pharmaceuticals, in addition to the clarification of physiological activities of IL-18, an expedited establishment of mass production and characterization of IL-18 receptor (hereinafter abbreviated as "IL-18R") have been in great expectation.

Summary of the Invention

In view of the foregoing, the first object of this invention is to provide a polypeptide as IL-18R which can be easily prepared on a large scale.

The second object of this invention is to provide uses of such polypeptide as pharmaceuticals.

The third object of this invention is to provide a DNA which encodes the polypeptide.

The fourth object of this invention is to provide a process to prepare the polypeptide.

The fifth object of this invention is to provide an agent to neutralize IL-18 using the polypeptide.

The sixth object of this invention is to provide a method to neutralize IL-18 using the polypeptide.

We energetically and extensively screened various means which might attain these objects, eventually resulting in the finding that a substance which recognized IL-18 was present in L428 cell, a type of lymphoblastoid cell derived from a patient with Hodgkin's disease. We isolated and characterized this substance, revealing that its nature was proteinaceous, as well as that it well recognized and bound IL-18 even when in isolated form. It was also found that the IL-18R thus identified was efficacious in treatment and prevention of various diseases resulting from excessive immunoreaction, such as autoimmune diseases, because in mammals including human, IL-18R recognized and neutralized IL-18 which activated immune system. Further, we have energetically studied L428 cell using as probe some partial amino acid sequences of the IL-18R, resulting in obtainment of a DNA which did encode IL-18R. We confirmed that a polypeptide obtained by bringing such DNAs into expression in artificial manner well recognized IL-18 and shared some essential physiological activities with the IL-18R separated from L428 cell, as well as that it was preparable in desired amounts by recombinant DNA techniques using such DNA. Thus we accomplished this invention.

More particularly, this invention attains the first object with a polypeptide as IL-18R, which is obtainable through gene expression.

This invention attains the second object with an agent for IL-18R susceptive diseases, which contains as effective ingredient such polypeptide.

This invention attains the third object with a DNA which encodes the polypeptide.

This invention attains the forth object with a process to prepare polypeptide, comprising bringing into expression a DNA which encodes the polypeptide, and collecting the resultant polypeptide.

This invention attains the fifth object with an agent to neutralize IL-18, which contains as effective ingredient the

polypeptide.

This invention attains the sixth object with a method to neutralize IL-18, characterized by allowing the polypeptide to act on IL-18.

L428 cell, which is feasible in this invention, have been deposited in the Patent Microorganism Depository, National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, 1-3, Higashi 1 chome, Tsukuba-shi, Ibaraki-ken, 305, Japan, under the accession number of "FERM BP-5777" on and after December 24th, 1996.

Brief Explanation of the Accompanying Drawings

FIG. 1 shows that the monoclonal antibody MAb #117-10C binds to L428 cells and IL-18R while competing with IL-18.

FIG. 2 is an image of intermediate tone given on display, which shows IL-18R on gel electrophoresis visualized by the Western blotting method using the monoclonal antibody MAb #117-10C.

FIG. 3 shows the inhibitory action of the monoclonal antibody MAb #117-10C on the activity of IL-18.

FIG. 4 is the chromatogram obtained by applying to IL-18R an immunoaffinity chromatography using the monoclonal antibody MAb #117-10C.

FIG. 5 is the peptide map of IL-18R.

FIG. 6 shows the structure of the recombinant DNA "pcDNA/HuIL-18R" of this invention.

FIG. 7 shows the structure of the recombinant DNA "pEFHIL18R-14" of this invention.

FIG. 8 shows the structure of the recombinant DNA "pEFHIL18RD1-2-H" of this invention.

FIG. 9 shows the structure of the recombinant DNA "pEFHIL18RD1-H" of this invention.

FIG. 10 shows the structure of the recombinant DNA "pEFMIL18RSHT" of this invention.

Throughout the Figures, the symbol "Pcmv" indicates the cytomegalo virus promotor; "EF1αP", the elongation factor promotor; "IL-18R cDNA", the cDNA encoding the polypeptide of this invention; "EFHIL18R-14 cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; "HIL18RD1-2-H cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; "HIL18RD1-H cDNA", the cDNA encoding the soluble polypeptide of human origin according to this invention; and "EFMIL18RSHT cDNA", the cDNA encoding the soluble polypeptide of mouse origin according to this invention.

Detailed Description of the Invention

This invention relates to a polypeptide as IL-18R, which is obtainable through gene expression. The polypeptide of human origin according to this invention usually contains as partial amino acid sequence(s) one or more amino acid sequences of SEQ ID NOs:12 to 19: As a whole, it contains a part or whole of the amino acid sequence of SEQ ID NO: 20. While the polypeptide of mouse origin according to this invention usually contains a part or whole of the amino acid sequence of SEQ ID NO:21. Thus, the wording "polypeptide" as referred to in this invention shall include, in addition to those which wholly contain the amino acid sequence of either SEQ ID NO:20 or 21, for example, those which contain the same amino acid sequence but with addition of one or more amino acids, in particular, those which contain one or more amino acids linked to the C-and/or N-termini in SEQ ID NO:20 or 21; those which contain the same amino acid sequence as in SEQ ID NOs:20 and 21 but with deletion of one or more amino acids, in particular, soluble polypeptides which contain the amino acid sequences of SEQ ID NOs:22 to 25; and those which contain either of the amino acid sequences as described above but with a saccharide chain, as far as they are obtainable through gene expression and possess the essential functions of IL-18R. As to IL-18, those of human and mouse origins commonly consisting of 157 amino acids have been documented: Human IL-18 bears the amino acid sequence of SEQ ID NO:26 (where the amino acid with symbol "Xaa" represents either isoleucine or threonine), while mouse counterpart, the amino acid sequence of SEQ ID NO:27 (where the amino acid with symbol "Xaa" represents either methionine or threonine).

The polypeptide of this invention is usually prepared by applying recombinant DNA techniques, more particularly, by bringing into expression in artificial manner a DNA which encodes the polypeptide, and collecting the resultant polypeptide. This invention provides, in addition to a DNA which encodes the polypeptide, a process to prepare the polypeptide using recombinant DNA techniques: By practicing such a process according to this invention, desired amounts the polypeptide can be easily obtained.

The DNA which is used in this invention are those which originating natural sources, those which can be obtained by artificially modifying them and those which can be obtained through chemical synthesis, provided that they do encode the polypeptide. Generally, in this field, in case of artificially expressing DNAs which encode polypeptides, one may replace one or more nucleotides in the DNAs with different nucleotides and/or link an appropriate nucleotide sequence to the DNAs, with purpose of improving their expression efficiency and/or the physiological and physicochemical properties of the polypeptides. Such modifications are feasible in the DNA of this invention of course: For

example, one can link to the 5′-and 3′-termini of the DNA as described above recognition sites for appropriate restriction enzymes, initiation and termination codons, promotors and/or enhancers, as far as the final polypeptide products do retain desired physiological activities. Thus, the wording "DNA" as referred to in this invention shall mean, in addition to those which encode any polypeptides as described above, those which are complementary thereto, and further those where one or more nucleotides have been replaced with different nucleotides while conserving the amino acid sequence.

To obtain such a DNA from natural sources, for example, mammalian cells including epithelial cells, endothelial cells, interstitial cells, chondrocytes, monocytes, granulocytes, lymphocytes neurocytes and their established cell lined of human and mouse origins are screened with oligonucleotides as probe which can be prepared with reference to the amino acid sequences of SEQ ID NOs;12 to 25. Examples of preferred cells are cell lines which are obtained by establishing hemopoietic cells including lymphocytes, in particular, JM cells, HDLM-2 cells, MOLT-16 cells and PEER cells described in Jun Minowada, *Cancer Review*, Vol.10, pp.1-18 (1988), and lymphoblastoid cells such as L428 cell (FERM BP-5777), KG-1 cell (ATCC CCL-246) and U-937 cells (ATCC CRL-1593.2). The human and mouse DNAs obtained in this way usually contain a part or whole of respective nucleotide sequences of SEQ ID NOs:1 and 2. For example, as shown in SEQ ID NO:7, the DNA obtained from L428 cell, a type of lymphoblastoid cell derived from a patient with Hodgkin's disease, consists of the nucleotide sequence of SEQ ID NO:1 encoding the amino acid sequence of SEQ ID NO:20, and another nucleotide sequence encoding signal peptide which is linked to the 5′-terminal in the nucleotide sequence of the SEQ ID NO:1. Soluble polypeptides with the amino acid sequences of SEQ ID NOs:22 to 25 are usually encoded by respective nucleotide sequences of SEQ ID NOs:3 to 6, which are usually used in a form where, as shown in the nucleotide sequences of SEQ ID NOs:8 to 11, a nucleotide sequence encoding signa1 peptide is linked to the 5′-terminal in the nucleotide sequences of SEQ ID NOs:3 to 6. Such a DNA can be also obtained through usual chemical synthesis, and in any case, DNAs can be amplified to desired levels by PCR method once they become available. By the way, the amino acid sequences of SEQ ID NOs:20 and 21 are described along with the amino acid sequences for signal peptides in P. Parnet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3,967-3,970 (1996): This paper however makes neither suggestion nor teaching that the polypeptides with the amino acid sequences of SEQ ID NOs:20 and 21 do function as IL-18R.

Such DNA expresses the polypeptide when introduced into an appropriate host of microbe, animal or plant origin. The DNA of this invention is usually prepared into a recombinant DNA prior to introduction into host. Such recombinant DNA, which consists of the DNA of this invention and an autonomously replicable vector, can be easily prepared with usual recombinant DNA techniques, provided that the DNA is available. Examples of vectors which can receive the DNA of this invention are plasmid vectors including pKK223-3, pCDNAI/Amp, BCMGSNeo, pcDL-SRα, pKY4, pCDM8, pCEV4, pME18S and pEF-BOS. Autonomously replicable vectors usually comprises other nucleotide sequences, for example, promotor, enhancer, replication origin, terminator of transcription, splicing sequence and/or selection marker which facilitate the expression of the DNA of this invention in particular hosts. Expression of the DNA becomes artificially regulatable upon external stimuli when it is used in combination with either heat shock protein promotor or interferon-α promotor as disclosed in Japanese Patent Kokai No. 163,368/95 by the same applicant.

Conventional methods are feasible in the insertion of the DNA of this invention into such vector. More particularly, a gene with the DNA of this invention and an autonomously replicable vector are first digested with restriction enzyme and/or ultrasonication, then the resultant DNA and vector fragments are ligated. Ligation of DNA and vector fragments become much easier when genes and vectors are digested with restriction enzymes specific to particular nucleotides, for example, *Acc*I, *Bam*HI, *Bst*XI, *Eco*RI, *Hin*dIII, *Not*I, *Pst*I, *Sac*I, *Sal*I, *Sma*I, *Spe*I, *Xba*I and *Xho*I. To ligate DNA and vector fragments, they are first annealed, if necessary, then exposed to DNA ligase *in vivo* or *in vitro*. The recombinant DNA thus obtained is unlimitedly replicable in hosts of microbe and animal origins.

Such recombinant DNA is introduced into an appropriate host, prior to use in preparation of the polypeptide. Although conventional hosts of microbe, animal and plant origins are feasible in this invention, it is preferable to choose a host of yeast or mammalian origin in case that the final use of the polypeptide is pharmaceuticals. Examples of host cells of mammalian origin are epithelial cell, interstitial cell and hemopoietic cell of human, monkey, mouse and hamster origins, in particular, 3T3 cell (ATCC CCL-92), C127I cell (ATCC CRL-1616), CHO-K1 cell (ATCC CCL-61), CV-1 cell (ATCC CCL-70), COS-1 cell (ATCC CRL-1650), HeLa cell (ATCC CCL-2), MOP-8 cell (ATCC CRL-1709) and their mutant strains. To introduce the DNA of this invention into such a host, one can employ conventional methods, for example, DEAE-dextran method, calcium phosphate transfection method, electroporation method, lipofection method, microinjection method and viral infection method using retrovirus, adenovirus, herpesvirus and vaccinia virus. To select among the resultant transformants a clone which is capable of producing the polypeptide, the transformants are cultivated on culture medium, followed by selecting one or more clones where production of the polypeptide is observed. Recombinant DNA techniques using host cells of mammalian origin are detailed, for example, *Jikken-Igaku-Bessatsu, Saibo-Kogaku Handbook* (The handbook for the cell engineering), edited by Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA, published by Yodosha. Co., Ltd., Tokyo, Japan (1992), and *Jikken-Igaku-Bessatsu, Biomanual Series 3, Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), edited by Takashi YOKOTA and

Kenichi ARAI, published by Yodosha Co., Ltd., Tokyo, Japan (1993).

The transformant thus obtained produces and secretes the polypeptide inside and/or outside the host cell when cultivated on culture medium. Such cultivation is feasible with conventional culture media directed to cultivation of transformants, which are usually composed by adding to a bufferized water as base inorganic ions such as sodium ion, potassium ion, calcium ion, phosphoric ion and chloric ion; minor elements, carbon sources, nitrogen sources, amino acids and vitamins which meet to the metabolism of particular hosts; and, if necessary, sera, hormones, cell growth factors and cell adhesion factors. Particular media are, for example, 199 medium, DMEM medium, Ham's F12 medium, IMDM medium, MCDB 104 medium, MCDB 153 medium, MEM medium, RD medium, RITC 80-7 medium, RPMI-1630 medium, RPMI-1640 medium and WAJC 404 medium. One can obtain a culture product containing the polypeptide by inoculating on such a culture medium a transformant in an amount of $1x10^4$-$1x10^7$ cells/ml, preferably, $1x10^5$-$1x10^6$ cells/ml, and subjecting the transformant to suspension or monolayer culture at around 37°C for 1 day to 1 week, preferably, 2 to 4 days while replacing the culture medium with a fresh preparation, if necessary. The culture product thus obtained usually contains about 1 μg/l to 1 mg/l polypeptide, dependently of the type of transformant and cultivation conditions.

The culture product obtained in this way is first subjected to ultrasonication, cell-lytic enzyme and/or detergent to disrupt cells, if necessary, then polypeptides are separated from the cells or cell debris by filtration and centrifugation, followed by purification. In the purification, a culture product which has been separated from cell or cell debris is subjected to conventional methods common in purification of biologically-active proteins, for example, salting-out, dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatography, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis and isoelectric focusing gel electrophoresis which are used in combination, if necessary. The purified polypeptide is then concentrated and lyophilized into liquid or solid to meet to its final use. The IL-18 and monoclonal antibody, disclosed in Japanese Patent Kokai No.193,098/96 and Japanese Patent Application No.356,426/96 by the same applicant, are very useful in purification of the polypeptide: Immunoaffinity chromatographies using these do yield a high-purity preparation of the polypeptide with minimized costs and labors.

The polypeptide of this invention exhibits a remarkable efficacy in treatment and prevention of various diseases resulting from excessive immunoreaction because in mammals including human, the polypeptide recognizes and binds IL-18 which may activate immune system. Immune system, which is in nature to defend living bodies from harmful foreign substances, may cause unfavorable results in living bodies because of its nature. When mammals receive a graft of organ, for example, skin, kidney, liver, heart and bone marrow, the rejection reaction and immunoreaction against alloantigen may activate T-cells, resulting in the occurrence of inflammation and proliferation of lymphocytes. Similar phenomena are observed in case that host receives the invasion by heteroantigens, for example, allergens, which are not recognized as self. In autoimmune diseases, allergic reactions are induced by substances which must be recognized as self. The polypeptide of this invention exhibits a remarkable efficacy in treatment and prevention of various diseases resulting from such an immunoreaction because the polypeptide suppresses or regulates the immunoreaction when administered in mammals including human. Thus, the wording "susceptive diseases" as referred to in this invention shall mean all the diseases resulting from augmented immunoreaction which can be treated and/or prevented by the direct or indirect action of IL-18R: Particular susceptive diseases are, for example, rejection reactions associated with a graft of organ as described above, autoimmune and allergic diseases including pernicious anemia, atrophic gastritis, insulin-resistant diabetes, Wegener granulomatosis, discoid lupus erythematosus, ulcerative colitis, cold agglutinin-relating diseases, Goodpasture's syndrome, primary biliary cirrhosis, sympathetic ophtalmitis, hyperthyroidism, juvenile onset type diabetes, Sjögren syndrome, autoimmune hepatitis, autoimmune hemolytic anemia, myasthenia gravis, systemic scleroderma, systemic lupus erythematosus, polyleptic cold hemoglobinuria, polymyositis, periarteritis nodosa, multiple sclerosis, Addison's disease, purpura hemorrhagica, Basedow's disease, leukopenia, Behçet's disease, climacterium praecox, rheumatoid arthritis, rheumatopyra, chronic thyroiditis, Hodgkin's disease, HIV-infections, asthma, atopic dermatitis, allergic nasitis, pollinosis and apitoxin-allergy. In addition, the polypeptide of this invention is efficacious in treatment and prevention of septic shock which results from production or administration of excessive IFN-γ.

Thus, the agent for susceptive disease, which contains as effective ingredient the polypeptides of this invention, would find a variety of uses as anti-autoimmune-diseases, anti-allergies, anti-inflammatories, immunosuppressants, hematopoietics, leukopoietics, thrombopoietics, analgesics and antipyretics directed to treatment and/or prevention of susceptive diseases as illustrated in the above. The agent according to this invention is usually prepared into liquid, suspension, paste and solid forms which contain the polypeptide in an amount of 0.00001-100 w/w %, preferably, 0.0001-20 w/w %, dependently on the forms of agents as well as on the types and symptoms of susceptive disease.

The agent for susceptive diseases according to this invention includes those which are solely composed of the polypeptide, as well as including those in composition with, for example, one or more physiologically-acceptable carriers, excipients, diluents, adjuvants, stabilizers and, if necessary, other biologically-active substances: Examples of such stabilizer are proteins such as serum albumins and gelatin; saccharides such as glucose, sucrose, lactose, maltose,

trehalose, sorbitol, maltitol, mannitol and lactitol; and buffers which are mainly composed of phosphate or succinate. Examples of the biologically-active substances usable in combination are FK506, glucocorticoid, cyclophosphamide, nitrogen mustard, triethylenethiophosphoramide, busulfan, pheniramine mustard, chlorambucil, azathioprine, 6-mercaptopurine, 6-thioguanine, 6-azaguanine, 8-azaguanine, 5-fluorouracil, cytarabine, methotrexate, aminopterin, mitomycin C, daunorubicin hydrochloride, actinomycin D, chromomycin A_3, bleomycin hydrochloride, doxorubicin hydrochloride, cyclosporin A, L-asparaginase, vincristine, vinblastine, hydroxyurea, procarbazine hydrochloride, adrenocortical hormone and auri colloid; receptor antagonists to cytokines other than IL-18, for example, antibodies respectively against interleukin-1 receptor protein, interleukin-2 receptor protein, interleukin-5 receptor protein, interleukin-6 receptor protein, interleukin-8 receptor protein and interleukin-12 receptor protein; and antagonists respectively against TNF-α receptor, TNF-β receptor, interleukin-1 receptor, interleukin-5 receptor and interleukin-8 receptor.

The agent for susceptive diseases according to this invention includes pharmaceuticals in minimal dose unit: The wording "pharmaceutical in minimal dose unit" represents those which are prepared into a physically united form suitable for prescription and also allowed to contain the polypeptide in an amount corresponding to its single dose or multiple (up to 4-fold) or divisor (up to 1/40) thereof: Examples of such form are injection, liquid, powder, granule, tablet, capsule, sublingual, ophthalmic solution, nasal drop and suppository. The agent for susceptive diseases according to this invention can be administrated through both oral and parenteral routes to exhibit in each case a remarkable efficacy in treatment and prevention of susceptive diseases. More particularly, the polypeptide is administered through oral route or parenteral route such as intradermal, subcutaneous, intramuscular or intravenous route at a dose of about 1 μg/time/adult to about 1g/time/adult, preferably, about 10 μg/time/adult to about 100 mg/time/adult 1 to 4 times/day or 1 to 5 times/week over 1 day to 1 year.

The DNA which encodes the polypeptide of this invention is useful in "gene therapies". Particularly, in usual gene therapies, the DNA of this invention is first inserted in a vector derived from virus such as retrovirus, adenovirus or adeno-associated virus and, alternatively, embedded in either cationic- or membrane fusible-liposomes, then the inserted or embedded DNA is directly injected in a patient with an IL-18 susceptive disease and, alternatively, introduced into lymphocytes, which have been collected from the patient, and implanted in the patient. In adoptive immuno gene therapies, by introducing the DNA of this invention into effector cells similarly as in the usual gene therapies, the cytotoxicity of effector cells against tumors and virus-infected cells is enhanced and this would strengthen adoptive immunotherapy. In tumor vaccine gene therapy, tumor cells, which have been extracted from a patient, are introduced with the DNA of this invention similarly as in the usual gene therapies, allowed to proliferate in vitro to a prescribed level and then self-transplanted to the patient: The transplanted tumor cells act as vaccine in the body of the patient, exhibiting a strong and antigen-specific antitumor immunity. Thus, the DNA of this invention exhibits a remarkable efficacy in gene therapies for various diseases including, for example, malignant tumors, vial diseases, infections and autoimmune diseases, as well as in suppression of rejection reaction and excessive immunoreaction associated with grafts of organs and allergic diseases. General procedures for gene therapies are detailed in *Jikken-Igaku-Bessatsu, Biomanual UP Series, Idenshichiryo-no-Kisogijutsu* (Basic techniques for the gene therapy), edited by Takashi SHIMADA, Izumi SAITO, and Keiya OZAWA, published by Yodosha Co., Ltd., Tokyo, Japan (1996).

Further, the polypeptide of this invention is useful in affinity chromatography and labelled assay directed to purification and detection of IL-18 because the polypeptide bears properties of recognizing and binding IL-18. In addition, the polypeptide of this invention, in particular, that in soluble form is useful in screening *in vivo* or *in vitro* agonists and antagonists to IL-18. Furthermore, the agent to neutralize IL-18 containing as effective ingredient the polypeptide and the method to neutralize IL-18 where IL-18 is exposed to the polypeptide are useful in treatment of various diseases which result from production and administration of excessive IL-18.

The following Examples are to illustrate the way of practicing this invention. The techniques employed in Examples 1 to 3 are common in this field as detailed, for example, *Jikken-Igaku-Bessatsu, Saibo-Kogaku Handbook* (The handbook for the cell engineering), edited by Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA, published by Yodosha. Co., Ltd., Tokyo, Japan (1992), and *Jikken-Igaku-Bessatsu, Biomanual Series 3, Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), edited by Takashi YOKOTA and Kenichi ARAI, published by Yodosha Co., Ltd., Tokyo, Japan (1993).

Example 1

Preparation and characterization of IL-18R

Example 1-1

Preparation of IL-18R

Newborn hamsters were intraperitoneally injected with an anti-lymphocyte antibody of rabbit origin to suppress

their possible immunoreaction, subcutaneously injected at their dorsal areas with about $5x10^5$ cell/animal of L428 cells (FERM BP-5777), a type of lymphoblastoid cell derived from a patient with Hodgkin's disease, and fed in usual manner for 3 weeks. The tumor masses, subcutaneously occurred, about 10g each, were extracted, disaggregated and washed in usual manner in serum-free RPMI-1640 medium (pH 7.4), thus obtaining proliferated cells.

The proliferated cells were added with a mixture solution (volume ratio of 9:1) of 0.83 w/v % NH_4Cl and 170mM Tris-HCl buffer (pH 7.7) in an amount 10-fold larger than the wet weight of the cells, stirred and collected by centrifugation at 2,000rpm for 10 minutes. The cells were then suspended in an appropriate amount of phosphate buffered saline (hereinafter abbreviated as "PBS"), stirred, collected by centrifugation at 2,000rpm, resuspended to give a cell density of about $1x10^8$ cells/ml in 10mM Tris-HCl buffer (pH 7.2) with 1mM $MgCl_2$ and disrupted with "POLYTRON", a cell disrupter commercialized by Kinematica AG, Littau/Lucerne, Switzerland. The resultant was added with 10mM Tris-HCl buffer (pH 7.2) containing both 1mM $MgCl_2$ and 1M sucrose to give a final sucrose concentration of 0.2M, and centrifuged at 1,000rpm to collect the supernatant which was then centrifuged at 25,000rpm for 60 minutes, followed by collecting the precipitate. The precipitate was added with adequate amounts of 12mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (hereinafter abbreviated as "CHAPS"), 10mM ethylenediaminetetraacetatic acid (hereinafter abbreviated as "EDTA") and 1mM phenylmethylsulfonylfluoride, stirred at 4°C for 16 hours, and centrifuged at 25,000rpm for 60 min, followed by collecting the supernatant.

The supernatant was charged to a column of "WHEAT GERM LECTIN SEPHAROSE 6B", a gel product for affinity chromatography commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, pre-equilibrated in PBS with 12mM CHAPS, and the column was washed with PBS containing 12mM CHAPS, and then charged with PBS containing both 0.5 M N-acetyl-D-glucosamine and 12mM CHAPS while monitoring the protein content in the eluate with the absorbance of ultraviolet at a wave length of 280nm. The fractions with an absorbance of 0.16-0.20 were collected and pooled, thus obtaining about 25 liters of aqueous solution with a protein content of about 1 mg/ml per 10^{12} starting cells.

A small portion of the solution was sampled, added with 4ng human IL-18 which had been ^{125}I-labelled in usual manner, incubated at 4°C for 1 hour, added with appropriate amounts of "POLYETHYLENE GLYCOL 6000", a polyethylene glycol preparation with an averaged molecular weight of 6,000 daltons, commercialized by E. Merck, Postfach, Germany, and allowed to stand under ice-chilling conditions for 30 minutes to effect binding reaction. The reaction product was centrifuged at 6,000rpm for 5 minutes and the resultant precipitate was collected to determine the level of radioactivity. In parallel, there was provided another sections as control in which 3μg non-labelled human IL-18 was used along with ^{125}I-labelled human IL-18 and treated similarly as above. Comparison with control revealed that the radioactivity of the precipitate from the sample solution was significantly higher. This indicated that the aqueous solution obtained in the above did contain IL-18R and the I-18R recognized and bound IL-18 when exposed to IL-18.

Example 1-2

Binding ability to monoclonal antibody

L428 cells (FERM BP-5777) were suspended in RPMI-1640 medium (pH7.4), supplemented with 0.1 v/v % bovine serum albumin and also containing 0.1 v/v % NaN_3, to give a cell density of $4x10^7$ cells/ml, while monoclonal antibody MAb#117-10C specific to human IL-18R, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, was dissolved in another preparation of RPMI-1640 medium supplemented with 0.1 w/v% bovine serum albumin to give different concentrations of 0.019 μg/ml, 0.209 μg/ml, 2.3 μg/ml, 25.3 μg/ml and 139.5 μg/ml.

Fifty microliter aliquots of the cell suspension prepared in the above were mixed with 50μl of either solution with different monoclonal antibody concentrations, agitated at 4°C for 2 hours, added with 50μl of RPMI-1640 medium supplemented with 0.1 v/v % bovine serum albumin and also containing 4ng ^{125}I-labelled human IL-18 prepared in usual manner, and agitated at the same temperature for an additional 30 minutes. Subsequently, each cell suspension was added with 200μl mixture solution (volume ratio 1:1) of dibutylphthalate and diocthylphtalate and centrifuged at 10,000rpm and 20°C for 5 minutes, followed by collecting the resultant precipitates containing the cells which were then determined for radioactivity using "MODEL ARC-300", a gamma-ray counter commercialized by Aloka Co., Ltd, Tokyo, Japan.

In parallel, there were provided additional two sections where the monoclonal antibody was neglected, while 4ng ^{125}I-labelled human IL-18 was treated similarly as in the sample testing section with or without 4 micrograms of non-labelled human IL-18 (hereinafter referred to as "non-specific binding section" and "whole binding section" respectively). The levels of radioactivity found in "non-specific binding section" and "whole binding section" were put in Formula 1 together with that found in the sample testing section to calculate percent inhibition. The results were as shown in FIG. 1.

$$\text{Percent Inhibition} = \frac{\text{(Whole binding) - (Testing)}}{\text{(Whole binding) - (Non-specific binding)}} \times 100 \qquad \text{Formula 1}$$

Fifty microliter aliquots of an IL-18R in aqueous solution obtained by the method in Example 1-1 were added with 50μl solution with different concentrations for monoclonal antibody MAb #117-10C prepared similarly as above, agitated at 4°C for 2 hours, added with 4ng ^{125}I-labelled human IL-18, and agitated at 4°C for an additional 30 minutes. Subsequently, each mixture was added with 50μl of 4 mg/ml γ-globulin, allowed to stand under ice-chilling conditions for 30 minutes, added with 250μl of PBS with 20 w/v % polyethylene glycol, allowed to stand under ice-chilling conditions for an additional 30 minutes, and centrifuged at 6,000rpm at 4°C for 5 minutes, followed by collecting the resultant precipitates which were then determined for radioactivity similarly as above.

At the same time, there were provided additional two sections where the monoclonal antibody was neglected, while 4ng of ^{125}I-labelled human IL-18 were treated similarly as in the sample testing section with or without 4μg of non-labelled human IL-18 (hereinafter referred to as "whole binding section" and "non-specific binding section" respectively). The levels of radioactivity found in these two section were put in Formula 1 together in that found in the sample testing section to calculate percent inhibition. The results were as shown in FIG.1.

As seen in FIG. 1, in both cases of using L428 cell and IL-18R in solution, the binding of IL-18 to L428 cell and IL-18R were inhibited much more as the concentration of monoclonal antibody MAb #117-10C elevated. This indicated that the monoclonal antibody MAb #117-10C was bound to the possible IL-18R on the surface of L428 cell in a fashion competing with IL-18, as well as that the aqueous solution obtained by the method in Example 1 did contain a protein capable of recognizing IL-18 or IL-18R and the monoclonal antibody MAb #117-10C specifically reacted with the IL-18R.

Example 1-3

Western blotting

A portion of the IL-18R in aqueous solution obtained by the method in Example 1 was sampled, added with 2/3 volume of a mixture solution of 2.5 w/v % sodium dodecyl sulfate and 50 v/v % glycerol, incubated at 37°C for 1 hour, and separated into respective proteinaceous components on conventional SDS-PAGE using 10-20% gradient gel but using no reducing agent. The proteinaceous components on the gel were transferred in usual manner to a nitrocellulose membrane which was then soaked for 1 hour in an appropriate amount of 50mM Tris-HCl buffer (pH7.5) with 10μg/ml of monoclonal antibody MAb #117-10C obtained by the methods described in Japanese Patent Application No.356,426/96 by the same applicant, 10 v/v % "BLOCK ACE", an immobilizing agent commercialized by Dainippon Seiyaku Co., Ltd., Osaka, Japan, and 0.05 v/v % "TWEEN 20", a detergent commercialized by City Chemical Corp., New York, U.S.A., and washed in 50mM Tris-HCl buffer (pH7.5) with 0.05 v/v % Tween 20 to remove the remaining antibody. The membrane was then soaked in Tris-HCl buffer (pH 7.5) with an appropriate amount of an anti-mouse immunoglobulin antibody of rabbit origin prelabelled with horse radish peroxidase, 10 v/v % "BLOCK ACE" and 0.05 v/v % "TWEEN 20" for 1 hour to effect reaction, washed in 50mM Tris-HCl buffer (pH 7.5) with 0.05 v/v % "TWEEN 20" and developed using "ECL kit", a kit for development commercialized by Amersham Corp., Arlington Heights, U.S.A.

At the same time, there was provided another section without the monoclonal antibody MAb #117-10C as control and it was treated similarly as above. The molecular weight markers were bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (30,000 daltons), trypsin inhibitor (20,100 daltons) and α-lactoalbumin (14,000 daltons). The results were as shown in FIG. 2.

In the gel electrophoresis in FIG. 2, Lane 2 (with monoclonal antibody) bore a distinct band of IL-18R which was never found in Lane 3 (without monoclonal antibody).

Example 1-4

Inhibition of IL-18 activity

KG-1 cells (ATCC CCL246), an established cell line derived from a patient with acute myelogenous leukemia, were suspended in RPMI-1640 medium (pH 7.2), supplemented with 10 v/v % fetal bovine serum and also containing 100μg/ml kanamycin and 18.8mM Na_2HPO_4, to give a cell density of 1×10^7 cells/ml, added with monoclonal antibody MAb #117-10C, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, to give a concentration of 10μg/ml and incubated at 37°C for 30 minutes.

The KG-1 cells in suspension were distributed on 96-well microplate to give respective amounts of 50μl/well, added with 50μl of human IL-18 which had been dissolved in a fresh preparation of the same medium to give respective con-

centrations of 0ng/ml, 1.56ng/ml, 3.12ng/ml, 6.25ng/ml, 12.5ng/ml and 25ng/ml, further added with 50μl/well of 5μg/ml lipopolysaccharide in a fresh preparation of the above medium, and incubated at 37°C for 24 hours, after which each supernatant was collected and determined for IFN-γ content by conventional enzyme immunoassay. In parallel, there were provided additional sections without the monoclonal antibody MAb #117-10C for respective IL-18 concentrations as control and they were treated similarly as above. The results were as shown in FIG. 3. The IFN-γ contents in FIG. 3 were calibrated with reference to the standardized IFN-γ preparation Gg23-901-530 available from the International Institute of Health, USA, and expressed in the International Unit(IU).

The results in FIG. 3 indicated that the presence of monoclonal antibody MAb #117-10C inhibited the induction of IFN-γ by IL-18 in KG-1 cell as immunocompetent cell. This also indicated that monoclonal antibody MAb #117-10C blocked the IL-18R on the surface of KG-1 cell in a fashion competing with Il-18, thus preventing the signal transduction of IL-18 to KG-1 cell.

Example 1-5

Purification of IL-18R

Seventy-eight milligrams of a monoclonal antibody MAb #117-10C, obtained by the method described in Japanese Patent Application No.356,426/96 by the same applicant, was dissolved in an appropriate amount of distilled water and the solution was dialyzed against borate buffer (pH 8.5) with 0.5M NaCl at 4°C for 16 hours. Thereafter, in usual manner, an appropriate amount of "CNBr-ACTIVATED SEPHAROSE 4B", a CNBr-activated gel, commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, was added to the dialyzed solution and allowed to react at 4°C for 18 hours under gentle stirring conditions to immobilize the monoclonal antibody MAb #117-10C on the gel.

The gel was packed into column in a plastic cylinder, equilibrated with 2mM CHAPS, charged with an IL-18R in aqueous solution obtained by the method in Example 1-1, and applied with PBS with 12mM CHAPS to remove non-adsorbed components. The column was then applied with 35mM ethylamine containing 2mM CHAPS (pH 10.8) while collecting the eluate in every 8ml fractions which were then checked for presence of IL-18R by the method in Example 1-1 using ^{125}I-labelled human IL-18. The chromatogram obtained in this operation was as shown in FIG.4.

As seen in FIG. 4, IL-18R was eluted in a single sharp peak when immunoaffinity chromatography using monoclonal antibody MAb #117-10C was applied to a mixture of IL-18R and contaminants such as the aqueous solution of IL-18R in Example 1-1. The fractions corresponding to this single peak were collected, pooled and lyophilized, thus obtaining a purified IL-18R in solid form.

Thereafter, a portion of the purified IL-18R was sampled, incubated in PBS at 100°C for 5 minutes, and determined for residual activity by the method in Example 1-2, resulting in no binding to IL-18 which proved that IL-18R was inactivated by heating. This would support that the nature of this receptor is proteinaceous.

Further, a portion of the purified IL-18R obtained in the above was dissolved in an appropriate amount of PBS, dialyzed against PBS at ambient temperature overnight, added with an appropriate amount of ^{125}I-labelled human IL-18 prepared by the method in Example 1-1 and 1mM "BS^3", a polymerizing agent commercialized by Pierce, Rockford, U.S.A., and allowed to stand at 0°C for 2 hours to form a conjugate of IL-18R and ^{125}I-labelled human IL-18. The reaction mixture was added with Tris-HCl buffer (pH7.5), allowed to stand at 0°C for an additional 1 hour to suspend the conjugation reaction, separated into respective proteinaceous components on SDS-PAGE using a set of molecular weight markers and dithiothreitol as reducing agent, and subjected to autoradiogram analysis.

The apparent molecular weight for this conjugate of IL-18R and ^{125}I-labelled human IL-18 was about 50,000 to 200,000 daltons when estimated with reference to the mobility of molecular weight markers on the autoradiogram. Since the molecular weight of IL-18 is about 20,000 daltons, the molecular weight of IL-18R can be estimated about 30,000-180,000 daltons on the assumption that IL-18R binds one human IL-18 molecule.

Example 1-6

Peptide mapping of IL-18R

A purified IL-18R obtained by the method in Example 1-5 was electrophoresed on SDS-PAGE using 7.5 w/v % gel with 2 w/v % dithiothreitol as reducing agent, and the gel was then soaked for 5 minutes in a mixture solution of 40 v/v % aqueous methanol and 1 v/v % acetic acid with 0.1 w/v % Coomassie Brilliant Blue for development, and soaked for an additional 2 hours for destaining in the same solution but without Coomassie Brilliant Blue, after which the stained part in the gel, molecular weight of 80,000-110,000 daltons, was cut off, added with 50 v/v % aqueous acetonitrile containing 0.2 M $(NH_4)_2CO_3$ and repeatedly agitated at ambient temperature. Thereafter, the gel slices were lyophilized, added with 0.2M $(NH_4)_2CO_3$ (pH 8.0), allowed to stand for 5 minutes to effect swelling, added with appropriate amounts of 1mM hydrochloric acid with 0.1μg/μl "SEQUENCING GRADE MODIFIED TRYPSIN", a reagent of trypsin commer-

cialized by Promega Corp., Madison, U.S.A., and 0.2 M $(NH_4)_2CO_3$ (pH 8.9), and allowed to react at 37°C overnight. After suspending with 10 v/v % aqueous acetic acid solution, the reaction mixture was added with a mixture solution of 0.1 v/v % trifluoroacetic acid and 60 v/v % aqueous acetonitrile and agitated at ambient temperature, after which the resultant supernatant was collected, concentrated *in vacuo* and centrifugally filtered, thus obtaining a concentrate with peptide fragments.

The concentrate was charged to "μRPC C2/C18 SC2.1/10", a column for high-performance liquid chromatography commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, pre-equilibrated with 0.065 v/v % trifluoroacetic acid, and then applied at a flow rate of 100μl/min with 0.055 v/v % trifluoroacetic acid containing 80 v/v % aqueous acetonitrile under liner gradient of acetonitrile increasing from 0 to 80 v/v % over 160 minutes immediately after application of the eluent. While monitoring the absorbance at a wavelength of 240nm, the eluate was fractioned to separately collect respective peptide fragments which eluted about 45, 50, 55, 58, 62, 72, 75 and 77 minutes after application of the eluent. The peptide fragments (hereinafter referred to as "peptide fragment 1", "peptide fragment 2", "peptide fragment 3", "peptide fragment 4", "peptide fragment 5", "peptide fragment 6", "peptide fragment 7" and "peptide fragment 8" in the order of elution) were analyzed in usual manner for amino acid sequence using "MODEL 473A", a protein sequencer commercialized by Perkin-Elmer Corp., Norwalk, U.S.A, revealing that the peptide fragments 1 to 8 bore the amino acid sequences of SEQ ID NOs:12 to 19 respectively. The peptide map obtained by this operation was as shown in FIG.5.

Example 2

Preparation of DNA

Example 2-1

Preparation of total RNA

In usual manner, L428 cells (FERM BP-5777) were suspended in RPMI-1640 medium (pH7.2) supplemented with 10 v/v % fetal bovine serum, and proliferated at 37°C while scaling up the cultivation. When the cell density reached a prescribed level, the proliferated cells were collected, suspended in 10mM sodium citrate (pH7.0) containing both 6M guanidine isothiocyanate and 0.5 w/v% sodium N-laurylsarcosinate, and then disrupted with a homogenizer.

Aliquots of 0.1M EDTA (pH 7.5) containing 5.7M $CsCl_2$ were placed in 35ml-reaction tubes, poured with the cell disruptant obtained in the above in layer over the EDTA in each tube, and subjected to ultracentrifugation at 20°C at 25,000rpm for 20 hours to collect the RNA fraction. The RNA fraction was distributed in 15ml-centrifugation tubes, added with an equivolume each of a mixture solution of chloroform/1-butanol (volume ratio 4:1), agitated for 5 minutes and centrifuged at 4°C at 10,000rpm for 10 minutes, after which the aqueous layer was collected, added with 2.5-fold volume of ethanol and allowed to stand at 20°C for 2 hours to precipitate the total RNA. The precipitate was collected, washed with 75 v/v % aqueous ethanol, and then dissolved in 0.5ml of sterilized distilled water to obtain a solution of the total RNA originating from L428 cell.

Example 2-2

Preparation of mRNA

An aqueous solution containing total RNA solution obtained by the method in Example 2-1 was added with 0.5ml of 10mM Tris-HCl buffer (pH 7.5), containing both 1mM EDTA and 0.1 w/v % sodium N-laurylsarcosinate, to bring the total volume to 1 ml. The mixture solution was added with 1 ml of "OLIGOTEX™-dT30 〈SUPER〉", a latex with an oligonucleotide of $(dT)_{30}$ commercialized by Japan Roche K. K., Tokyo, Japan, reacted at 65°C for 5 minutes and rapidly cooled in an ice-chlling bath. Thereafter, the reaction mixture was added with 0.2ml of 5mM NaCl, incubated at 37°C for 10 minutes, centrifuged at 10,000rpm for 10 minutes to collect the resultant precipitate in pellet form which was then suspended in 0.5ml of sterilized distilled water and incubated at 65°C for 5 minutes to desorb the mRNA from the latex. The obtained solution was added with an appropriate amount of ethanol, and the resultant precipitate was collected and lyophilized to obtain a solid of mRNA.

Example 2-3

Preparation of DNA fragment encoding polypeptide

Four microliters of 25mM $MgCl_2$, 2μl of 100mM Tris-HCl buffer (pH 8.3) containing 500mM KCl, 1μl of 25mM dNTP

mix, 0.5 μl of 40units/μl ribonuclease inhibitor and 1μl of 200units/μl reverse transcriptase were placed in a 0.5ml-reaction tube, added with 10 ng of an mRNA, obtained by the method in Example 2-2, along with an appropriate amount of random hexanucleotides, and added with sterilized distilled water to bring the total volume of 20μl. The obtained mixture was incubated first at 42°C for 20 minutes, then at 99°C for 5 minutes to suspend the reaction, thus obtaining a reaction mixture containing a first strand cDNA.

Twenty microliters of the reaction mixture was added with 1μl of 2.5 units/μl "CLONED Pfu POLYMERASE", a DNA polymerase commercialized by Stratagene Cloning Systems, California, U.S.A., 10μl of the reaction buffer and 1μl of 25mM dNTP mix, both commercialized by Stratagene Cloning Systems, added with 0.1μg each of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5′-TCAGTCGACGCCACCATGAATTGTAGAGAA-3′ and 5′-GAAGCGGCCGCATCATTAAGACTCGGAAAGAAC-3′ which had been prepared on the basis of the amino acid sequence described in P. Parnet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3967-3970 (1996), added with sterile distilled water to bring the total volume to 100μl. The resultant mixture was subjected first to 3-time cycles of incubating at 95°C for 1 minute, 42°C for 2 minutes and 72°C for 3 minutes in the given order, then to 35-time cycles of incubating at 95°C for 1 minute, 60°C for 2 minutes and 72°C for 3 minutes in the given order to effect PCR reaction.

Fifty nanograms of the obtained PCR product was added with 1 ng of "pCR-Script Cam SK(+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, U.S.A., and then subjected to ligation reaction at 16°C for 2 hours using "DNA LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Syuzo, Co., Ltd., Otsu, Shiga, Japan, to insert the DNA fragment of the PCR product in the plasmid vector. A portion of the reaction product was sampled and used in usual manner to transform "XL1-BLUE MRF′ KAN", an *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, U.S.A.

Example 3

Preparation of recombinant DNA

A transformant obtained by the method in Example 2-3 was inoculated in LB medium containing 30μg/ml chloramphenicol and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. After confirming by the dideoxy method that the plasmid DNA contained the nucleotide sequence of SEQ ID NO:7, the plasmid DNA was exposed to both restriction enzymes *Not*I and *Sal*I, and 100 ng of the obtained DNA fragment was added with 10ng of "pcDNAI/Amp", a plasmid vector with a modified multiple cloning site, commercialized by Invitrogen Corporation, San Diego, U.S.A., which had been predigested with both restriction enzymes *Not*I and *Xho*I, and subjected to ligation reaction at 16°C for 2 hours using "LIGATION KIT VERSION 2", a ligation kit commercialized by Takara Syuzo Co., Ltd., Otsu, Shiga, Japan. A portion of the reaction product was sampled and introduced in usual manner into "XL1-BLUE MRF′ KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant "cDNA/HuIL-18R" which contained a recombinant DNA "pcDNA/HuIL-18R" of this invention. The recombinant DNA "pcDNA/HuIL-18R" was analyzed in usual manner, revealing that in the recombinant DNA, a DNA "IL-18R cDNA", which contained the nucleotide sequence of SEQ ID NO:1 encoding the polypeptide of this invention, was linked downstream the cytomegalo virus promotor Pcmv, as shown in FIG. 6.

Example 4

Preparation of transformant

A transformant "cDNA/HuIL-18R" obtained by the method in Example 3 was inoculated in LB medium (pH 7.5) containing 100μg/ml ampicillin and cultured at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. Separately, COS-1 cell (ATCC CRL-1650), a fibroblastic cell line derived from a kidney of African green monkey was proliferated in usual manner, and 20 micrograms of the plasmid DNA obtained in the above was introduced by conventional electroporation method into 1×10^7 COS-1 cells to obtain transformant cells which contained the DNA of this invention.

Example 5

Preparation of polypeptide

DMEM medium (pH 7.2) supplemented with 10 v/v % fetal bovine serum was distributed in flat-bottomed culture bottles, inoculated with transformant cells, obtained by the method in Example 4, to give a cell density of 1×10^5

cells/ml, and cultured at 37°C in 5 v/v % CO_2 incubator for 3 days. After removing the supernatant from the culture, PBS containing both 5mM EDTA and 0.02 w/v % NaN_3 was placed in the culture bottles to desorb the proliferated cells.

After washing in PBS, the proliferated cells were rinsed in a buffer containing 20mM HEPES, 10mM KCl, 1.5mM $MgCl_2$ and 0.1mM EDTA (hereinafter referred to as "hypotonic buffer"), and suspended in a fresh preparation of the hypotonic buffer to give a cell density of 2×10^7 cells/ml. The cell suspension was homogenized with a Dounce-type homogenizer under ice-chilling conditions, and the resultant homogenate was centrifuged at 15,000rpm at 5 minutes to remove both cell nuclei and intact cells, and dialyzed overnight against PBS containing 2mM CHAPS.

The dialyzed product was charged to a column of immobilized monoclonal antibody MAb #117-10C, prepared by the method in Example 1-5, which was then applied with PBS containing 12mM CHAPS to remove non-adsorbed components. Thereafter, the column was applied with 35 mM ethylamine (pH10.8) containing 2 mM CHAPS while collecting and fractionating the eluate. was applied to the column, and the eluate was fractionally collected. Each fraction was then checked for presence of the polypeptide of human origin by the method in Example 1-1 using ^{125}I-labelled human IL-18, selected and pooled to obtain per 10^8 starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:20. The protein content in the solution was about 10μg/ml.

The polypeptide thus obtained was studied for physicochemical properties by the methods in Example 1. As the result, the polypeptide obtained in this Example contained each amino acid sequence in SEQ ID NOs:12 to 19 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of the IL-18R from L428 cell.

Example 6

Soluble polypeptide from human origin

Example 6-1

Preparation of recombinant DNA

One nanogram of a recombinant DNA "pcDNA/HuIL-18R" obtained by the method in Example 3, 10μl of 10×PCR buffer and 1μl of 25mM dNTP mix were placed in 0.5ml-reaction tube, added with 1 microliter of 2. units/microliter Pfu DNA polymerase, added with appropriate amounts of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5′-TCAGTCGACGCCACCATGAATTGTAGAGAATTA-3′ and 5′-GAAGCGGCCGCATCATTATCTTGTGAAGACGTG-3′, and with sterile distilled water to bring the total volume to 100μl. The resultant mixture was subjected first to 3-time cycles of incubating at 94°C for 1 minute, 42°C for 2 minutes and 72°C for 3 minutes in the given order, then to 35-time cycles of incubating at 94°C for 1 minute, 60°C for 2 minutes and 72°C for 3 minutes in the given order to effect PCR reaction.

Fifty nanograms of the obtained PCR product was added with 1ng of "pCR-SCRIPT SK(+)", a plasmid vector commercialized by Takara Syuzo Co. Ltd., Otsu, Shiga, Japan, and reacted using "DNA LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Shuzo Co. Ltd., Otsu, Shiga, Japan, at 16°C for 2 hours to insert the DNA fragment as the PCR product into the plasmid vector. A portion of the reaction product was sampled and "XL1-BLUE MRF′ KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., was transformed therewith in usual manner.

The transformant obtained in the above was inoculated in LB medium (pH 7.5) containing 100μg/ml ampicillin and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. After confirming by the dideoxy method that the plasmid DNA contained the nucleotide sequence of SEQ ID NO:10, the plasmid DNA was exposed to both restriction enzymes *Not*I and *Sal*I, and 100 ng of the resultant DNA fragment was added with 10ng of "pEF-BOS", a plasmid vector prepared in accordance with the method described in S. Mizushima, *Nucleic Acid Research*, Vol.18, No.17, pp.5,332 (1990) with slight modification and also predigested with both restriction enzymes *Not*I and *Xho*I, and subjected to ligation reaction using "LIGATION KIT VERSION 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Otsu, Shiga, Japan, at 16°C for 2 hours. A portion of the reaction product was sampled and introduced in usual manner into "XL1-BLUE MRF′ KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., thus obtaining a transformant "EFHIL18R-14" which contained a recombinant DNA "pEFHIL18R-14" of this invention. The recombinant DNA "pEFHIL18R-14" was analyzed in usual manner, revealing that in the recombinant DNA, a cDNA "EFHIL18R-14 cDNA", which contained the nucleotide sequence of SEQ ID NO:6 encoding the polypeptide of this invention, was located downstream the elongation factor 1 promotor EF1αP as shown in FIG. 7.

Example 6-2

Preparation of transformant

A transformant "EFHIL18R-14" obtained by the method in Example 6-1 was inoculated in LB medium (pH 7.5) containing 100μg/ml ampicillin and cultivated at 37°C for 18 hours, after which the cells were collected from the culture and treated in usual manner to obtain the plasmid DNA. Separately, COS-1 cell (ATCC CRL-1650), a fibroblastoid cell line derived from a kidney of African green monkey, was proliferated in usual manner, and 20 micrograms of the plasmid DNA obtained in the above was introduced by conventional electroporation method into 1×10^7 COS-1 cells to obtain transformant cells which contained the DNA of this invention.

Example 6-3

Preparation of soluble polypeptide

"ASF104", a serum-free nutrient culture medium commercialized by Ajinomoto Co., Inc., Tokyo, Japan, was distributed in flat-bottomed culture bottles, inoculated with ransformant cells, obtained by the method in Example 6-2, to givee a cell density of 1×10^5 cells/ml, and cultured in usual manner at 37°C in 5 v/v % CO_2 incubator for 3 days. The supernatant was collected from the culture and charged to a column of an immobilized monoclonal antibody #117-10C prepared by the method in Example 1-5, after which the column was applied first with PBS containing 12mM CHAPS to remove non-adsorbed components, then with 35mM ethylamine (pH 10.8) containing 2 mM CHAPS while collecting and fractionating the eluate. Each fraction was checked for presence of human soluble polypeptide by the method in Example 1-1 using ^{125}I-labelled human IL-18, selected and pooled to obtain per 10^8 starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:22. The protein content in the solution was about 10μg/ml.

The soluble polypeptide thus obtained was studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained each amino acid sequences in SEQ ID NOs:12 to 17 and 19 as partial sequences, as well as exhibiting physiological activities which were similar to the IL-18R from L428 cell.

Example 7

Soluble polypeptide of human origin

One nanogram of an recombinant DNA "pEFHIL18R-14" obtained by the method in Example 6-1, 10μl of 10xPCR buffer and 1μl of 25mM dNTP mix were placed in 0.5ml-reaction tube, added with 1μl of 2.5units/μl Pfu DNA polymerase, further added with appropriate amounts of oligonucleotides as sense and antisense primers having respective nucleotide sequences as shown with 5′-TCAGTCGACGCCACCATGAATTGTAGAG-3′ and 5′-GAAGCGGCCGCTCATTAGTGATGGTGATGGTGATGTGCAACATGGTTAAGCTT-3′, and filled up to 100μl with sterile distilled water. The resultant mixture was subjected first to 3-time cycles of incubating at 94°C for 1 minute, 42°C for 2 minutes and 72°C for 1 minute in the given order, then to 35-time cycles of incubating at 94°C for 1 minute, 64°C for 1 minute and 72°C for 1 minute in the given order to effect PCR reaction, thus obtaining a DNA fragment which consisted of the nucleotide sequence of SEQ ID NO:5, a digestion site for restriction enzyme *Sal*I and a Kozak's sequence both linked to the 5′-terminal of the nucleotide sequence of SEQ ID NO:5, and a digestion site for restriction enzyme *Not*I and a nucleotide sequence encoding $(His)_6$ tag both linked to the 3′-terminal of the nucleotide sequence of SEQ ID NO:5. This DNA fragment was introduced similarly as in Example 6-1 in "XL1-Blue MRF'Kan", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant which contained a recombinant DNA "pEFHIL18RD1-2-H" according to this invention. Analysis of the recombinant DNA in usual manner confirmed that in this recombinant DNA a cDNA "HIL18RD1-2-H", which contained the nucleotide sequence of SEQ ID NO:5 encoding the polypeptide of this invention, was located downstream the elongation factor promotor EF1αP as shown in FIG. 8.

The recombinant DNA "pEFHIL18RD1-2-H" was introduced in COS-1 cells similarly as in Example 6-2 using the transformant thus obtained, and the COS-1 cells were then cultivated similarly as in Example 6-3. The supernatant of the resultant culture was concentrated with membrane filtration, and charged on a column of "Ni-NTA Spin Kit", a gel product for affinity chromatography commercialized by QIAGEN GmbH, Hilden, Germany, which was then applied with PBS containing 20mM imidazole to remove the non-adsorbed fractions. Thereafter, the column was applied with PBS containing 250mM imidazole, and the eluate was collected in fractions while checking the presence of human soluble polypeptide in each fraction by the method in Example 1-1 using ^{125}I-labelled human IL-18, after which the fractions

with the polypeptide were collected and pooled, thus obtaining about 2ml of an aqueous solution containing the polypeptide with the amino acid sequence of SEQ ID NO:23 per starting 10^8 cells. The protein content in the solution was about 10μg/ml.

The soluble polypeptide thus obtained was studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained a part or whole of each amino acid sequences in SEQ ID NOs:14 to 16 and 19 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of IL-18R from L428 cell.

Example 8

Soluble polypeptide of human origin

A transformant containing a recombinant DNA "pEFHIL18RD1-H" according to this invention was prepared similarly as in Example 7, except that sense and antisense primers were replaced with oligonucleotides having respective nucleotide sequences as shown with 5′-TCAGTCGACGCCACCATGAATTGTAGAG-3′ and GAAGCGGCCGCTCATTAGTGATGGTGATGGTGATGTCTTTCAGTGAAACAGCT-3′. Analysis of the recombinant DNA in usual manner confirmed that in the recombinant DNA a cDNA "HIL18RD1-H", which contained the nucleotide sequence of SEQ ID NO:3 encoding the polypeptide of this invention, was located downstream the elongation factor promotor EF1αP as shown in FIG. 9. Thereafter, similarly as in Example 7, the recombinant DNA was introduced in COS-1 cells and brought into expression, thus obtaining about 2ml of an aqueous solution containing a polypeptide with the amino acid sequence of SEQ ID NO:24 per 10^8 starting cells. The protein content in the solution was about 10μg/ml.

The polypeptide of this invention thus obtained were studied for physicochemical properties by the method in Example 1. As the result, the soluble polypeptide obtained in this Example contained each amino acid sequences of SEQ ID NOs:14 and 15 as partial amino acid sequences, as well as exhibiting physiological activities which were similar to those of the IL-18R from L428 cell.

Example 9

Soluble polypeptide of mouse origin

Example 9-1

Preparation of recombinant DNA

A reaction product containing a first strand cDNA was obtained by subjecting an mRNA, prepared in usual manner from mouse liver, in place with that from L428 cell to the same reaction to synthesize first strand cDNA as in Example 2-3. The reaction product was treated by the same PCR method as in Example 2-3, except that the sense and antisense primers were replaced with oligonucleotides having respective nucleotide sequence as shown with 5′-TCAGTCGACGCCACCATGCATCATGAAGAA-3′ and 5′-GAAGCGGCCGCATCATTAGTGATGGTGATGGTGATGTGTAAAGACATGGCC-3′, which had been prepared on the basis of the amino acid sequence described in P. Parnet et al., *The Journal of Biological Chemistry*, Vol.271, pp.3,967-3,970 (1996) and also the nucleotide sequence of SEQ ID NO:1: This operation gave a DNA fragment which comprised the nucleotide sequence of SEQ ID NO:11, a digestion site for restriction enzyme *Sal*I linked to the 5′-terminal in the nucleotide sequence of the SEQ ID NO:11, and a cleavage site for restriction enzyme *Not*I and a nucleotide sequence encoding $(His)_6$ tag both linked to the 3′-terminal in the nucleotide sequence of the SEQ ID NO:11.

According to the method in Example 6-1, this DNA fragment was introduced into "XL1-BLUE MRF′ KAN", a strain of *Escherichia coli* commercialized by Stratagene Cloning Systems, California, U.S.A., to transform. After a plasmid DNA was collected from the transformant and confirmed to contain the nucleotide sequence of SEQ ID NO:11, the plasmid DNA was introduced into "XL1-BLUE MRF′ KAN", a strain of *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, U.S.A., to obtain a transformant "EFMIL18RSHT" which contains a recombinant DNA "pEFMIL18RSHT" according to this invention. Analysis in usual manner confirmed that in the recombinant DNA "pEFMIL18RSHT" a cDNA "EFMIL18RSHT cDNA", which contained the nucleotide sequence of SEQ ID NO:4 encoding the polypeptide of this invention, was linked to downstream of the elongation factor 1 promotor EF1αP, as shown in FIG. 8.

Example 9-2

Preparation of transformant and soluble polypeptide

According to the method in Example 6-2, a plasmid DNA was collected from a transformant "EFMIL18RSHT" obtained by the method in Example 9-1, and introduced into COS-1 cells to obtain transformant cells which contained a DNA encoding a soluble polypeptide of mouse origin.

"ASF104", a serum-free nutrient culture medium commercialized by Ajinomoto Co., Inc., Tokyo, Japan, was distributed in flat-bottomed culture bottles, inoculated with the transformed COS-1 cells to give a cell density of 1×10^5cells/ml, and cultivated in usual manner at 37°C in 5 v/v % CO_2 incubator for 3 days. The supernatant was collected from the resultant culture and charged to a column of "Ni-NTA", a gel product for affinity chromatography, commercialized by QIAGEN GmbH, Hilden, Germany, after which the column was applied first with PBS containing 20mM imidazole to remove non-adsorbed components, then with PBS containing 250mM imidazole while collecting and fractionating the eluate. The fractions were checked for presence of mouse soluble polypeptide by the method in Example 1-1 using ^{125}I-labelled mouse IL-18, selected and pooled, thus obtaining per 10^8 starting cells about 2 ml of an aqueous solution which contained a polypeptide with the amino acid sequence of SEQ ID NO:25. The protein content in the solution was about 100μg/ml. The soluble polypeptide thus obtained was studied in accordance with the method in Example 1, revealing that it efficiently neutralized mouse IL-18.

Example 10

Liquid agent

Either polypeptide obtained by the method in Examples 5 to 8 was separately dissolved in aliquots of physiological saline containing as stabilizer 1 w/v % "TREHAOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, to give respective concentration of 1 mg/ml, and the resultant mixtures were separately and sterilely filtered with membrane in usual manner to obtain four distinct liquid agents.

The products, which are excellent in stability, are useful as injection, ophthalmic solution and collunarium in treatment and prevention of susceptive diseases including autoimmune diseases.

Example 11

Dried injection

One hundred milligrams of either polypeptide obtained by the methods in Example 5 to 8 was separately dissolved in aliquots of physiological saline containing 1 w/v % sucrose as stabilizer, the resultant solutions were separately and sterilely filtered with membrane, distributed in vials in every 1 ml aliquot, lyophilized and sealed in usual manner to obtain four distinct pulverized agents.

The products, which are excellent in stability, are useful as dried injection in treatment and prevention of susceptive diseases including autoimmune diseases.

Example 12

Ointment

"HI-BIS-WAKO 104", a carboxyvinylpolymer commercialized by Wako Pure Chemicals, Tokyo, Japan, and "TREHAOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were dissolved in sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and either polypeptide obtained by the methods in Examples 5 to 8 was separately mixed with aliquots of the resultant solution to homogeneity, and adjusted to pH7.2 to obtain four distinct paste agents containing about 1 mg/g of the polypeptide of this invention each.

The products, which are excellent in both spreadablity and stability, are useful as ointment in treatment and prevention of susceptive diseases including autoimmune diseases.

Example 13

Tablet

Aliquots of "FINETOSE", a pulverized anhydrous crystalline alpha-maltose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were separately admixed with either polypeptide, obtained by the methods in Examples 5 to 8, and aliquots of "LUMIN" as cell activator, [bis-4-(1-ethylquinoline)][γ-4′-(1-ethylquinoline)] pentamethionine cyanine, to homogeneity, and the resultant mixtures were separately tableted in usual manner to obtain four distinct types of tablets, about 200 mg each, containing about 1mg/tablet of the polypeptide of this invention and also 1mg/tablet of LUMIN each.

The products, which are excellent in swallowability and stability and also bears an cell activating property, are useful as tablet in treatment and prevention of susceptive diseases including autoimmune diseases.

Experiment

Acute toxicity test

In usual manner, a variety of agents, obtained by the methods in Examples 8 to 11, were percutaneously or orally administrated or intraperitoneally injected to 8 week-old mice. As the result, the LD_{50} of each sample was proved about 1 mg or higher per body weight of mouse in terms of the amount of the polypeptide, regardless of administration route. This does support that the polypeptide of this invention is safe when incorporated in pharmaceuticals directed to use in mammals including human.

As explained above, this invention is based on the discovery of a novel receptor protein which recognizes IL-18. The polypeptide of this invention exhibits a remarkable efficacy in relief of rejection reaction associated with grafts of organs and also in treatment and prevention of various disease resulting from excessive immunoreaction because the polypeptide bears properties of suppressing and regulating immunoreaction in mammals including human. Further, the polypeptide of this invention is useful in clarification of physiological activities of IL-18, establishment of hybridoma cells which are capable of producing monoclonal antibodies specific to IL-18R, and also affinity chromatography and labelled assay to purify and detect IL-18. In addition, the polypeptide of this invention, in particular, that in soluble form is useful in screening *in vivo* and *in vitro* agonists and antagonists to IL-18. The polypeptide of this invention, which bears these outstanding usefulness, can be easily prepared in desired amounts by the process according to this invention using recombinant DNA techniques.

This invention, which exhibits these remarkable effects, would be very significant and contributive to the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
NAME:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO

(ii) TITLE OF INVENTION:POLYPEPTIDES

(iii) NUMBER OF SEQUENCES:27

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(A) ADDRESSEE:KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO
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(C) CITY:OKAYAMA
(E) COUNTRY:JAPAN
(F) POSTAL CODE (ZIP):700

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE:Floppy disk
(B) COMPUTER:IBM PC compatible
(C) OPERATING SYSTEM:PC-DOS/MS-DOS

(vii) PRIOR APPLICATION DATA:
(A1) APPLICATION NUMBER: JP 74,697/97
(B1) FILING DATE:March 12, 1997

(vii) PRIOR APPLICATION DATA:
(A2) APPLICATION NUMBER: JP 215,488/97
(B2) FILING DATE:July 28, 1997

(vii) PRIOR APPLICATION DATA:
(A3) APPLICATION NUMBER: JP 291,837/97
(B3) FILING DATE:October 9, 1997

(2)INFORMATION FOR SEQ ID NO:1:
(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH:1563 base pairs
(B)TYPE:nucleic acid
(C)strandedness:double
(D)TOPOLOGY:linear
(ii)MOLECULE TYPE:cDNA
(ix)FEATURE:
(A)NAME/KEY:mat peptide
(B)LOCATION:1..1563
(C)IDENTIFICATION METHOD:E
(xi)SEQUENCE DESCRIPTION:SEQ ID NO:1:

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96
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
```

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          35                  40                  45
CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG
192
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
     50                  55                  60
GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA
240
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
 65                  70                  75                  80
ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT
288
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA ATT GTG GAA
336
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
           100                 105                 110
GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC TAT CAA ACA
384
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
      115                 120                 125
CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG CTA CTA CTG
432
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
 130                 135                 140
GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG TTT GAA GAT
480
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT GGA AAA CTA
528
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA GAT CGC AGT
576
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
           180                 185                 190
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624
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      195                 200                 205
GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG CTG AAT GAA
672
Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu
 210                 215                 220
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720
Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro
225                 230                 235                 240
AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA GAA GGC AAA
768
Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys
                245                 250                 255
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816
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CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA GGC ACA GAC
864
Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp
      275                 280                 285
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912
Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly
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CAC GTC TTC ACA AGA GGA ATG ATC ATA GCT GTT TTG ATC TTG GTG GCA
960
His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile Leu Val Ala
305                 310                 315                 320
GTA GTG TGC CTA GTG ACT GTG TGT GTC ATT TAT AGA GTT GAC TTG GTT
1008
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                325                 330                 335
CTA TTT TAT AGA CAT TTA ACG AGA AGA GAT GAA ACA TTA ACA GAT GGA
1056
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1104
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1152
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1200
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385                 390                 395                 400
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1296
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1344
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        435                 440                 445
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1392
Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr Asp Phe Thr Phe
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1440
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465                 470                 475                 480
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1488
Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp Lys Asn Leu
                485                 490                 495
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1536
Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg Asp Glu Pro
            500                 505                 510
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1563
Glu Val Leu Pro Val Leu Ser Glu Ser
        515                 520
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(3) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:1557 base pairs
(B) TYPE:nucleic acid
(C) strandedness:double
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:cDNA
(ix) FEATURE:
(A) NAME/KEY:mat peptide
(B) LOCATION:1..1557
(C) IDENTIFICATION METHOD:S
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

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96
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            20                  25                  30
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144
Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
        35                  40                  45
AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT GAT CAC
192
Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His
    50                  55                  60
ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG TAC ATT
240
Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
65                  70                  75                  80
TCT CAA GTC GGA AAT GAT CGT CGC AAT TGG ACC TTA AAT GTC ACC AAA
288
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336
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            100                 105                 110
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384
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        115                 120                 125
GAA GAG CTG ATC CAG GAC ACA TGG CTG TAT AAG AAC TGT AAG GAA ATA
432
Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile
    130                 135                 140
TCC AAA ACC CCA AGG ATC CTG AAG GAT GCC GAG TTT GGA GAT GAG GGC
480
Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
145                 150                 155                 160
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528
Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn
                165                 170                 175
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672
Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp
    210                 215                 220
CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT AAT GTG
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Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val
225                 230                 235                 240
CAA GAA GAC AGG AAG GAG ACG ACA ACA TGG ATT TCT GAA GGC AAA CTG
768
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864
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        275                 280                 285
AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA GGC CAT
912
Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
    290                 295                 300
GTC TTT ACA GGA GGA GTA ACT GTG CTT GTT CTC GCC TCT GTG GCA GCA
960
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305                 310                 315                 320
GTG TGT ATA GTG ATT TTG TGT GTC ATT TAT AAA GTT GAC TTG GTT CTG
1008
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                325                 330                 335
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1056
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            340                 345                 350
ACA TAT GAT GCC TTT GTG TCT TAC CTG AAA GAG TGT CAT CCT GAG AAT
1104
Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys His Pro Glu Asn
        355                 360                 365
AAA GAA GAG TAT ACT TTT GCT GTG GAG ACG TTA CCC AGG GTC CTG GAG
1152
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1200
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385                 390                 395                 400
GGC GGA GCT GTT GTC GAG GAG ATC CAT TCA CTG ATA GAG AAA AGC CGG
1248
Gly Gly Ala Val Val Glu Glu Ile His Ser Leu Ile Glu Lys Ser Arg
                405                 410                 415
AGG CTA ATC ATC GTT CTC AGC CAG AGT TAC CTG ACT AAC GGA GCC AGG
1296
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            420                 425                 430
CGT GAG CTC GAG AGT GGA CTC CAC GAA GCA CTG GTA GAG AGG AAG ATT
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1344
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        435                 440                 445
AAG ATC ATC TTA ATT GAG TTT ACT CCA GCC AGC AAC ATC ACC TTT CTC
1392
Lys Ile Ile Leu Ile Glu Phe Thr Pro Ala Ser Asn Ile Thr Phe Leu
    450                 455                 460
CCC CCG TCG CTG AAA CTC CTG AAG TCC TAC AGA GTT CTA AAA TGG AGG
1440
Pro Pro Ser Leu Lys Leu Leu Lys Ser Tyr Arg Val Leu Lys Trp Arg
465                 470                 475                 480
GCT GAC AGT CCC TCC ATG AAC TCA AGG TTC TGG AAG AAT CTT GTT TAC
1488
Ala Asp Ser Pro Ser Met Asn Ser Arg Phe Trp Lys Asn Leu Val Tyr
                485                 490                 495
CTG ATG CCC GCA AAA GCC GTC AAG CCA TGG AGA GAG GAG TCG GAG GCG
1536
Leu Met Pro Ala Lys Ala Val Lys Pro Trp Arg Glu Glu Ser Glu Ala
            500                 505                 510
CGG TCT GTT CTC TCA GCA CCT
1557
Arg Ser Val Leu Ser Ala Pro
        515
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(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:312 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(ix) FEATURE:

(A) NAME/KEY:mat peptide

(B) LOCATION:1..312

(C) IDENTIFICATION METHOD:S

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

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96
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
        35                  40                  45
CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG
192
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
    50                  55                  60
GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA
240
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
65                  70                  75                  80
ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT
288
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
```

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AAA CAC AGC TGT TTC ACT GAA AGA
312
Lys His Ser Cys Phe Thr Glu Arg
            100
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(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:921 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(ix) FEATURE:

(A) NAME/KEY:mat peptide

(B) LOCATION:1..921

(C) IDENTIFICATION METHOD:S

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

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  1               5                  10                  15
CCT TTT TAT CTG AAG CCA TGT GGC ATA TCT GCA CCA GTG CAC AGG AAT
96
Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn
            20                  25                  30
GAA ACA GCC ACC ATG AGA TGG TTC AAA GGC AGT GCT TCA CAT GAG TAT
144
Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
        35                  40                  45
AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT GAT CAC
192
Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His
    50                  55                  60
ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG TAC ATT
240
Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
65                  70                  75                  80
TCT CAA GTC GGA AAT GAT CGT CGC AAT TGG ACC TTA AAT GTC ACC AAA
288
Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys
                85                  90                  95
AGA AAC AAA CAC AGC TGT TTC TCT GAC AAG CTC GTG ACA AGC AGA GAT
336
Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp
            100                 105                 110
GTT GAA GTT AAC AAA TCT CTG CAT ATC ACT TGT AAG AAT CCT AAC TAT
384
Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr
        115                 120                 125
GAA GAG CTG ATC CAG GAC ACA TGG CTG TAT AAG AAC TGT AAG GAA ATA
432
Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile
    130                 135                 140
TCC AAA ACC CCA AGG ATC CTG AAG GAT GCC GAG TTT GGA GAT GAG GGC
480
Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
145                 150                 155                 160
TAC TAC TCC TGC GTG TTT TCT GTC CAC CAT AAT GGG ACA CGG TAC AAC
528
Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn
```

```
                    165                 170                 175
ATC ACC AAG ACT GTC AAT ATA ACA GTT ATT GAA GGA AGG AGT AAA GTA
576
Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val
                180                 185                 190
ACT CCA GCT ATT TTA GGA CCA AAG TGT GAG AAG GTT GGT GTA GAA CTA
624
Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu
        195                 200                 205
GGA AAG GAT GTG GAG TTG AAC TGC AGT GCT TCA TTG AAT AAA GAC GAT
672
Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp
    210                 215                 220
CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT AAT GTG
720
Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val
225                 230                 235                 240
CAA GAA GAC AGG AAG GAG ACG ACA ACA TGG ATT TCT GAA GGC AAA CTG
768
Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu
                245                 250                 255
CAT GCT TCA AAA ATA CTG AGA TTT CAG AAA ATT ACT GAA AAC TAT CTC
816
His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu
            260                 265                 270
AAT GTT TTA TAT AAT TGC ACC GTG GCC AAC GAA GAA GCC ATA GAC ACC
864
Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr
        275                 280                 285
AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA GGC CAT
912
Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
    290                 295                 300
GTC TTT ACA
921
Val Phe Thr
305
```

(6) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:621 base pairs
  - (B) TYPE:nucleic acid
  - (C) strandedness:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA
- (ix) FEATURE:
  - (A) NAME/KEY:mat peptide
  - (B) LOCATION:1..621
  - (C) IDENTIFICATION METHOD:S
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```
GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA GGG GAA CCT
48
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
  1                 5                  10                  15
TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG ATT GAA ACA
96
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144
```

```
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
          35                  40                  45
CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG
192
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
     50                  55                  60
GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA
240
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
 65                  70                  75                  80
ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT
288
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA ATT GTG GAA
336
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
           100                 105                 110
GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC TAT CAA ACA
384
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
      115                 120                 125
CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG CTA CTA CTG
432
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
 130                 135                 140
GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG TTT GAA GAT
480
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
GAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT GGA AAA CTA
528
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA GAT CGC AGT
576
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
           180                 185                 190
AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT GTT GCA
621
Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala
      195                 200                 205
```

(7) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:927 base pairs
  - (B) TYPE:nucleic acid
  - (C) strandedness:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA
- (ix) FEATURE:
  - (A) NAME/KEY:mat peptide
  - (B) LOCATION:1..927
  - (C) IDENTIFICATION METHOD:E
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

```
GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA GGG GAA CCT
48
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
  1                 5                  10                  15
TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG ATT GAA ACA
```

```
96
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
                 20                  25                  30
ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA CAT GTG GAG
144
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
         35                  40                  45
CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT TGT GTT TTG
192
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
     50                  55                  60
GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC TTT TTC CAA
240
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
 65                  70                  75                  80
ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC AGA AGA AAT
288
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                 85                  90                  95
AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA ATT GTG GAA
336
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
            100                 105                 110
GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC TAT CAA ACA
384
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
        115                 120                 125
CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG CTA CTA CTG
432
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
    130                 135                 140
GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG TTT GAA GAT
480
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT GGA AAA CTA
528
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA GAT CGC AGT
576
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
            180                 185                 190
AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT GTT GCA GTG
624
Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val
        195                 200                 205
GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG CTG AAT GAA
672
Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu
    210                 215                 220
GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA TCG GAT CCT
720
Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro
225                 230                 235                 240
AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA GAA GGC AAA
768
Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys
                245                 250                 255
TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT GAA AGC AAT
816
```

```
Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn
            260                 265                 270
CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA GGC ACA GAC
864
Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp
        275                 280                 285
ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT ATC CCA GGC
912
Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly
    290                 295                 300
CAC GTC TTC ACA AGA
927
His Val Phe Thr Arg
305
```

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH:1620 base pairs
- (B) TYPE:nucleic acid
- (C) strandedness:double
- (D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(vi) ORIGINAL SOURCE:
- (A) ORGANISM:lymphoblastoid cell derived from a patient with Hodgkin's disease
- (B) INDIVIDUAL ISOLATE:L428 (FERM BP-5777)

(ix) FEATURE:
- (A) NAME/KEY:sig peptide
- (B) LOCATION:1..57
- (C) IDENTIFICATION METHOD:E

(ix) FEATURE:
- (A) NAME/KEY:mat peptide
- (B) LOCATION:58..1620
- (C) IDENTIFICATION METHOD:E

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

```
ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
                -15                 -10                 -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
            1                   5                   10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
    15                  20                  25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
 30                  35                  40                  45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
                50                  55                  60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
            65                  70                  75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
```

```
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
          80                  85                  90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA
384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys
     95                 100                 105
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC
432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr
110                 115                 120                 125
TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG
480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys
                130                 135                 140
CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG
528
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu
            145                 150                 155
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT
576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn
        160                 165                 170
GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA
624
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu
    175                 180                 185
GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT
672
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His
190                 195                 200                 205
GTT GCA GTG GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG
720
Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu
                210                 215                 220
CTG AAT GAA GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA
768
Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly
            225                 230                 235
TCG GAT CCT AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA
816
Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro
        240                 245                 250
GAA GGC AAA TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT
864
Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly
    255                 260                 265
GAA AGC AAT CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA
912
Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly
270                 275                 280                 285
GGC ACA GAC ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT
960
Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp
                290                 295                 300
ATC CCA GGC CAC GTC TTC ACA AGA GGA ATG ATC ATA GCT GTT TTG ATC
1008
Ile Pro Gly His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile
            305                 310                 315
TTG GTG GCA GTA GTG TGC CTA GTG ACT GTG TGT GTC ATT TAT AGA GTT
1056
```

```
Leu Val Ala Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg Val
        320                 325                 330
GAC TTG GTT CTA TTT TAT AGA CAT TTA ACG AGA AGA GAT GAA ACA TTA
1104
Asp Leu Val Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr Leu
    335                 340                 345
ACA GAT GGA AAA ACA TAT GAT GCT TTT GTG TCT TAC CTA AAA GAA TGC
1152
Thr Asp Gly Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys
350                 355                 360                 365
CGA CCT GAA AAT GGA GAG GAG CAC ACC TTT GCT GTG GAG ATT TTG CCC
1200
Arg Pro Glu Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu Pro
                370                 375                 380
AGG GTG TTG GAG AAA CAT TTT GGG TAT AAG TTA TGC ATA TTT GAA AGG
1248
Arg Val Leu Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg
            385                 390                 395
GAT GTA GTG CCT GGA GGA GCT GTT GTT GAT GAA ATC CAC TCA CTG ATA
1296
Asp Val Val Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu Ile
        400                 405                 410
GAG AAA AGC CGA AGA CTA ATC ATT GTC CTA AGT AAA AGT TAT ATG TCT
1344
Glu Lys Ser Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met Ser
    415                 420                 425
AAT GAG GTC AGG TAT GAA CTT GAA AGT GGA CTC CAT GAA GCA TTG GTG
1392
Asn Glu Val Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val
430                 435                 440                 445
GAA AGA AAA ATT AAA ATA ATC TTA ATT GAA TTT ACA CCT GTT ACT GAC
1440
Glu Arg Lys Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr Asp
                450                 455                 460
TTC ACA TTC TTG CCC CAA TCA CTA AAG CTT TTG AAA TCT CAC AGA GTT
1488
Phe Thr Phe Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg Val
            465                 470                 475
CTG AAG TGG AAG GCC GAT AAA TCT CTT TCT TAT AAC TCA AGG TTC TGG
1536
Leu Lys Trp Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp
        480                 485                 490
AAG AAC CTT CTT TAC TTA ATG CCT GCA AAA ACA GTC AAG CCA GGT AGA
1584
Lys Asn Leu Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg
    495                 500                 505
GAC GAA CCG GAA GTC TTG CCT GTT CTT TCC GAG TCT
1620
Asp Glu Pro Glu Val Leu Pro Val Leu Ser Glu Ser
510                 515                 520
```

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:369 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:cDNA

(ix) FEATURE:

(A) NAME/KEY:sig peptide

(B) LOCATION:1..57
(C) IDENTIFICATION METHOD:S
(ix) FEATURE:
(A) NAME/KEY:mat peptide
(B) LOCATION:58..369
(C) IDENTIFICATION METHOD:S
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

```
ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
                -15                 -10                  -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
  1                   5                  10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
      15                  20                  25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
 30                  35                  40                  45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
                50                  55                  60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
            65                  70                  75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
        80                  85                  90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA
369
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg
    95                  100
```

(10) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:678 base pairs
(B) TYPE:nucleic acid
(C) strandedness:double
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:cDNA
(ix) FEATURE:
(A) NAME/KEY:sig peptide
(B) LOCATION:1..57
(C) IDENTIFICATION METHOD:S
(ix) FEATURE:
(A) NAME/KEY:mat peptide
(B) LOCATION:58..678
(C) IDENTIFICATION METHOD:S
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

```
ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
```

```
                    -15                 -10                  -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
                 1                   5                  10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
     15                  20                  25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
 30                  35                  40                  45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
                 50                  55                  60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
               65                  70                  75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
            80                  85                  90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA
384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys
         95                 100                 105
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC
432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr
110                 115                 120                 125
TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG
480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys
                130                 135                 140
CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG
528
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu
            145                 150                 155
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT
576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn
        160                 165                 170
GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA
624
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu
    175                 180                 185
GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT
672
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His
190                 195                 200                 205
GTT GCA
678
Val Ala
```

(11) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:984 base pairs

(B) TYPE:nucleic acid

(C) strandedness:double
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:cDNA
(ix) FEATURE:
(A) NAME/KEY:sig peptide
(B) LOCATION:1..57
(C) IDENTIFICATION METHOD:E
(ix) FEATURE:
(A) NAME/KEY:mat peptide
(B) LOCATION:58..984
(C) IDENTIFICATION METHOD:E
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

```
ATG AAT TGT AGA GAA TTA CCC TTG ACC CTT TGG GTG CTT ATA TCT GTA
48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val
                -15                 -10                  -5
AGC ACT GCA GAA TCT TGT ACT TCA CGT CCC CAC ATT ACT GTG GTT GAA
96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu
            1               5                  10
GGG GAA CCT TTC TAT CTG AAA CAT TGC TCG TGT TCA CTT GCA CAT GAG
144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu
     15                  20                  25
ATT GAA ACA ACC ACC AAA AGC TGG TAC AAA AGC AGT GGA TCA CAG GAA
192
Ile Glu Thr Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu
 30                  35                  40                  45
CAT GTG GAG CTG AAC CCA AGG AGT TCC TCG AGA ATT GCT TTG CAT GAT
240
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp
                50                  55                  60
TGT GTT TTG GAG TTT TGG CCA GTT GAG TTG AAT GAC ACA GGA TCT TAC
288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr
            65                  70                  75
TTT TTC CAA ATG AAA AAT TAT ACT CAG AAA TGG AAA TTA AAT GTC ATC
336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile
         80                  85                  90
AGA AGA AAT AAA CAC AGC TGT TTC ACT GAA AGA CAA GTA ACT AGT AAA
384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys
     95                  100                 105
ATT GTG GAA GTT AAA AAA TTT TTT CAG ATA ACC TGT GAA AAC AGT TAC
432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr
110                 115                 120                 125
TAT CAA ACA CTG GTC AAC AGC ACA TCA TTG TAT AAG AAC TGT AAA AAG
480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys
                130                 135                 140
CTA CTA CTG GAG AAC AAT AAA AAC CCA ACG ATA AAG AAG AAC GCC GAG
528
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu
            145                 150                 155
TTT GAA GAT CAG GGG TAT TAC TCC TGC GTG CAT TTC CTT CAT CAT AAT
576
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn
        160                 165                 170
```

```
GGA AAA CTA TTT AAT ATC ACC AAA ACC TTC AAT ATA ACA ATA GTG GAA
624
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu
    175                 180                 185
GAT CGC AGT AAT ATA GTT CCG GTT CTT CTT GGA CCA AAG CTT AAC CAT
672
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His
190                 195                 200                 205
GTT GCA GTG GAA TTA GGA AAA AAC GTA AGG CTC AAC TGC TCT GCT TTG
720
Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu
                210                 215                 220
CTG AAT GAA GAG GAT GTA ATT TAT TGG ATG TTC GGG GAA GAA AAT GGA
768
Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly
            225                 230                 235
TCG GAT CCT AAT ATA CAT GAA GAG AAA GAA ATG AGA ATT ATG ACT CCA
816
Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro
        240                 245                 250
GAA GGC AAA TGG CAT GCT TCA AAA GTA TTG AGA ATT GAA AAT ATT GGT
864
Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly
    255                 260                 265
GAA AGC AAT CTA AAT GTT TTA TAT AAT TGC ACT GTG GCC AGC ACG GGA
912
Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly
270                 275                 280                 285
GGC ACA GAC ACC AAA AGC TTC ATC TTG GTG AGA AAA GAC ATG GCT GAT
960
Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp
                290                 295                 300
ATC CCA GGC CAC GTC TTC ACA AGA
984
Ile Pro Gly His Val Phe Thr Arg
            305
```

(12) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:975 base pairs
  - (B) TYPE:nucleic acid
  - (C) strandedness:double
  - (D) TOPOLOGY:linear
- (ii) MOLECULE TYPE:cDNA
- (ix) FEATURE:
  - (A) NAME/KEY:sig peptide
  - (B) LOCATION:1..54
  - (C) IDENTIFICATION METHOD:S
- (ix) FEATURE:
  - (A) NAME/KEY:mat peptide
  - (B) LOCATION:55..975
  - (C) IDENTIFICATION METHOD:S
- (xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

```
ATG CAT CAT GAA GAA TTA ATC TTG ACA CTC TGC ATT CTC ATT GTT AAA
48
Met His His Glu Glu Leu Ile Leu Thr Leu Cys Ile Leu Ile Val Lys
            -15                 -10                 -5
AGT GCC TCA AAA AGT TGT ATT CAC CGA TCA CAA ATT CAT GTG GTA GAG
96
Ser Ala Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu
```

```
          1               5                  10
GGA GAA CCT TTT TAT CTG AAG CCA TGT GGC ATA TCT GCA CCA GTG CAC
144
Gly Glu Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His
 15                  20                  25                  30
AGG AAT GAA ACA GCC ACC ATG AGA TGG TTC AAA GGC AGT GCT TCA CAT
192
Arg Asn Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His
                 35                  40                  45
GAG TAT AGA GAG CTG AAC AAC AGA AGC TCG CCC AGA GTC ACT TTT CAT
240
Glu Tyr Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His
             50                  55                  60
GAT CAC ACC TTG GAA TTC TGG CCA GTT GAG ATG GAG GAT GAG GGA ACG
288
Asp His Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr
         65                  70                  75
TAC ATT TCT CAA GTC GGA AAT GAT CGT CGC AAT TGG ACC TTA AAT GTC
336
Tyr Ile Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val
     80                  85                  90
ACC AAA AGA AAC AAA CAC AGC TGT TTC TCT GAC AAG CTC GTG ACA AGC
384
Thr Lys Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser
 95                 100                 105                 110
AGA GAT GTT GAA GTT AAC AAA TCT CTG CAT ATC ACT TGT AAG AAT CCT
432
Arg Asp Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro
                115                 120                 125
AAC TAT GAA GAG CTG ATC CAG GAC ACA TGG CTG TAT AAG AAC TGT AAG
480
Asn Tyr Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys
            130                 135                 140
GAA ATA TCC AAA ACC CCA AGG ATC CTG AAG GAT GCC GAG TTT GGA GAT
528
Glu Ile Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp
        145                 150                 155
GAG GGC TAC TAC TCC TGC GTG TTT TCT GTC CAC CAT AAT GGG ACA CGG
576
Glu Gly Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg
    160                 165                 170
TAC AAC ATC ACC AAG ACT GTC AAT ATA ACA GTT ATT GAA GGA AGG AGT
624
Tyr Asn Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser
175                 180                 185                 190
AAA GTA ACT CCA GCT ATT TTA GGA CCA AAG TGT GAG AAG GTT GGT GTA
672
Lys Val Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val
                195                 200                 205
GAA CTA GGA AAG GAT GTG GAG TTG AAC TGC AGT GCT TCA TTG AAT AAA
720
Glu Leu Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys
            210                 215                 220
GAC GAT CTG TTT TAT TGG AGC ATC AGG AAA GAG GAC AGC TCA GAC CCT
768
Asp Asp Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro
        225                 230                 235
AAT GTG CAA GAA GAC AGG AAG GAG ACG ACA ACA TGG ATT TCT GAA GGC
816
Asn Val Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly
    240                 245                 250
```

```
AAA CTG CAT GCT TCA AAA ATA CTG AGA TTT CAG AAA ATT ACT GAA AAC
864
Lys Leu His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn
255                 260                 265                 270
TAT CTC AAT GTT TTA TAT AAT TGC ACC GTG GCC AAC GAA GAA GCC ATA
912
Tyr Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile
                275                 280                 285
GAC ACC AAG AGC TTC GTC TTG GTG AGA AAA GAA ATA CCT GAT ATC CCA
960
Asp Thr Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro
            290                 295                 300
GGC CAT GTC TTT ACA
975
Gly His Val Phe Thr
        305
```

(13) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:5

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

```
Trp His Ala Ser Lys
  1
```

(14) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:7

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

```
Ile Met Thr Pro Glu Gly Lys
  1               5
```

(15) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:13

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

```
Ser Ser Gly Ser Gln Glu His Val Glu Leu Asn Pro Arg
  1               5                  10
```

(16) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:4

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(v) FRAGMENT TYPE:internal fragment

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

Ser Trp Tyr Lys
1

(17) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:10
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(v) FRAGMENT TYPE:internal fragment
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

Leu Asn His Val Ala Val Glu Leu Gly Lys
1 5 10

(18) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:6
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(v) FRAGMENT TYPE:internal fragment
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

Ser Phe Ile Leu Val Arg
1 5

(19) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:15
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(v) FRAGMENT TYPE:internal fragment
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

Thr Val Lys Pro Gly Arg Asp Glu Pro Glu Val Leu Pro Val Leu
1 5 10 15

(20) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:11
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(v) FRAGMENT TYPE:internal fragment
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys
1 5 10

(21) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:521
(B) TYPE:amino acid
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
1 5 10 15

```
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
                20                  25                  30
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
            35                  40                  45
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
        50                  55                  60
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
65                  70                  75                  80
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
            100                 105                 110
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
        115                 120                 125
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
    130                 135                 140
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
            180                 185                 190
Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val
        195                 200                 205
Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu
    210                 215                 220
Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro
225                 230                 235                 240
Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys
                245                 250                 255
Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn
            260                 265                 270
Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp
        275                 280                 285
Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly
    290                 295                 300
His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu Ile Leu Val Ala
305                 310                 315                 320
Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg Val Asp Leu Val
                325                 330                 335
Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr Leu Thr Asp Gly
            340                 345                 350
Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys Arg Pro Glu
        355                 360                 365
Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu Pro Arg Val Leu
    370                 375                 380
Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val
385                 390                 395                 400
Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu Ile Glu Lys Ser
                405                 410                 415
Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met Ser Asn Glu Val
            420                 425                 430
Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val Glu Arg Lys
        435                 440                 445
Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr Asp Phe Thr Phe
    450                 455                 460
Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg Val Leu Lys Trp
465                 470                 475                 480
Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe Trp Lys Asn Leu
                485                 490                 495
Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly Arg Asp Glu Pro
```

```
                500                 505                 510
Glu Val Leu Pro Val Leu Ser Glu Ser
        515                 520
```

(22) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:519

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

```
Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu Gly Glu
  1               5                  10                  15
Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn
             20                  25                  30
Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
         35                  40                  45
Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His
     50                  55                  60
Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
 65                  70                  75                  80
Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys
                 85                  90                  95
Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp
            100                 105                 110
Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr
        115                 120                 125
Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile
    130                 135                 140
Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
145                 150                 155                 160
Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn
                165                 170                 175
Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val
            180                 185                 190
Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu
        195                 200                 205
Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp
    210                 215                 220
Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val
225                 230                 235                 240
Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu
                245                 250                 255
His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu
            260                 265                 270
Asn Val leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr
        275                 280                 285
Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
    290                 295                 300
Val Phe Thr Gly Gly Val Thr Val Leu Val Leu Ala Ser Val Ala Ala
305                 310                 315                 320
Val Cys Ile Val Ile Leu Cys Val Ile Tyr Lys Val Asp Leu Val Leu
                325                 330                 335
Phe Tyr Arg Arg Ile Ala Glu Arg Asp Glu Thr Leu Thr Asp Gly Lys
            340                 345                 350
Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu Cys His Pro Glu Asn
        355                 360                 365
Lys Glu Glu Tyr Thr Phe Ala Val Glu Thr Leu Pro Arg Val Leu Glu
    370                 375                 380
Lys Gln Phe Gly Tyr Lys Leu Cys Ile Phe Glu Arg Asp Val Val Pro
```

```
385                 390                 395                 400
Gly Gly Ala Val Val Glu Glu Ile His Ser Leu Ile Glu Lys Ser Arg
                405                 410                 415
Arg Leu Ile Ile Val Leu Ser Gln Ser Tyr Leu Thr Asn Gly Ala Arg
            420                 425                 430
Arg Glu Leu Glu Ser Gly Leu His Glu Ala Leu Val Glu Arg Lys Ile
        435                 440                 445
Lys Ile Ile Leu Ile Glu Phe Thr Pro Ala Ser Asn Ile Thr Phe Leu
    450                 455                 460
Pro Pro Ser Leu Lys Leu Leu Lys Ser Tyr Arg Val Leu Lys Trp Arg
465                 470                 475                 480
Ala Asp Ser Pro Ser Met Asn Ser Arg Phe Trp Lys Asn Leu Val Tyr
                485                 490                 495
Leu Met Pro Ala Lys Ala Val Lys Pro Trp Arg Glu Glu Ser Glu Ala
            500                 505                 510
Arg Ser Val Leu Ser Ala Pro
        515
```

(23) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:309

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

```
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
  1                 5                  10                  15
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
        35                  40                  45
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
    50                  55                  60
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
65                  70                  75                  80
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
            100                 105                 110
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
        115                 120                 125
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
    130                 135                 140
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
            180                 185                 190
Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala Val
        195                 200                 205
Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu Leu Asn Glu
    210                 215                 220
Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly Ser Asp Pro
225                 230                 235                 240
Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro Glu Gly Lys
                245                 250                 255
Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly Glu Ser Asn
            260                 265                 270
Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly Gly Thr Asp
```

```
        275                 280                 285
Thr Lys Ser Phe Ile Leu Val Arg Lys Asp Met Ala Asp Ile Pro Gly
    290                 295                 300
His Val Phe Thr Arg
305
```

(24) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:207

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

```
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
  1               5                  10                  15
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
        35                  40                  45
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
    50                  55                  60
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
65                  70                  75                  80
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys Ile Val Glu
            100                 105                 110
Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr Tyr Gln Thr
        115                 120                 125
Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys Leu Leu Leu
    130                 135                 140
Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu Phe Glu Asp
145                 150                 155                 160
Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn Gly Lys Leu
                165                 170                 175
Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu Asp Arg Ser
            180                 185                 190
Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His Val Ala
        195                 200                 205
```

(25) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:104

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

```
Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu Gly Glu Pro
  1               5                  10                  15
Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu Ile Glu Thr
            20                  25                  30
The Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu His Val Glu
        35                  40                  45
Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp Cys Val Leu
    50                  55                  60
Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr Phe Phe Gln
65                  70                  75                  80
Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile Arg Arg Asn
                85                  90                  95
```

```
Lys His Ser Cys Phe Thr Glu Arg
           100

(26)INFORMATION FOR SEQ ID NO:25:
     (i)SEQUENCE CHARACTERISTICS:
          (A)LENGTH:307
          (B)TYPE:amino acid
          (D)TOPOLOGY:linear
     (ii)MOLECULE TYPE:peptide
     (xi)SEQUENCE DESCRIPTION:SEQ ID NO:25:

Ser Lys Ser Cys Ile His Arg Ser Gln Ile His Val Val Glu Gly Glu
  1               5                  10                  15
Pro Phe Tyr Leu Lys Pro Cys Gly Ile Ser Ala Pro Val His Arg Asn
            20                  25                  30
Glu Thr Ala Thr Met Arg Trp Phe Lys Gly Ser Ala Ser His Glu Tyr
        35                  40                  45
Arg Glu Leu Asn Asn Arg Ser Ser Pro Arg Val Thr Phe His Asp His
    50                  55                  60
Thr Leu Glu Phe Trp Pro Val Glu Met Glu Asp Glu Gly Thr Tyr Ile
65                  70                  75                  80
Ser Gln Val Gly Asn Asp Arg Arg Asn Trp Thr Leu Asn Val Thr Lys
                85                  90                  95
Arg Asn Lys His Ser Cys Phe Ser Asp Lys Leu Val Thr Ser Arg Asp
            100                 105                 110
Val Glu Val Asn Lys Ser Leu His Ile Thr Cys Lys Asn Pro Asn Tyr
        115                 120                 125
Glu Glu Leu Ile Gln Asp Thr Trp Leu Tyr Lys Asn Cys Lys Glu Ile
    130                 135                 140
Ser Lys Thr Pro Arg Ile Leu Lys Asp Ala Glu Phe Gly Asp Glu Gly
145                 150                 155                 160
Tyr Tyr Ser Cys Val Phe Ser Val His His Asn Gly Thr Arg Tyr Asn
                165                 170                 175
Ile Thr Lys Thr Val Asn Ile Thr Val Ile Glu Gly Arg Ser Lys Val
            180                 185                 190
Thr Pro Ala Ile Leu Gly Pro Lys Cys Glu Lys Val Gly Val Glu Leu
        195                 200                 205
Gly Lys Asp Val Glu Leu Asn Cys Ser Ala Ser Leu Asn Lys Asp Asp
    210                 215                 220
Leu Phe Tyr Trp Ser Ile Arg Lys Glu Asp Ser Ser Asp Pro Asn Val
225                 230                 235                 240
Gln Glu Asp Arg Lys Glu Thr Thr Thr Trp Ile Ser Glu Gly Lys Leu
                245                 250                 255
His Ala Ser Lys Ile Leu Arg Phe Gln Lys Ile Thr Glu Asn Tyr Leu
            260                 265                 270
Asn Val Leu Tyr Asn Cys Thr Val Ala Asn Glu Glu Ala Ile Asp Thr
        275                 280                 285
Lys Ser Phe Val Leu Val Arg Lys Glu Ile Pro Asp Ile Pro Gly His
    290                 295                 300
Val Phe Thr
305

(27)INFORMATION FOR SEQ ID NO:26:
     (i)SEQUENCE CHARACTERISTICS:
          (A)LENGTH:157
          (B)TYPE:amino acid
          (D)TOPOLOGY:linear
     (ii)MOLECULE TYPE:peptide
     (xi)SEQUENCE DESCRIPTION:SEQ ID NO:26:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
```

```
  1               5                  10                  15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
            20                  25                  30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        35                  40                  45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60
Ser Val Lys Cys Glu Lys Ile Ser Xaa Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150                 155
```

(28) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:157

(B) TYPE:amino acid

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

```
Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn
  1               5                  10                  15
Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
            20                  25                  30
Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
        35                  40                  45
Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
    50                  55                  60
Val Lys Asp Ser Lys Xaa Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile
65                  70                  75                  80
Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
                85                  90                  95
Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
            100                 105                 110
Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
        115                 120                 125
Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
    130                 135                 140
Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
145                 150                 155
```

Claims

1. A polypeptide as interleukin-18 receptor, which is obtainable through gene expression.

2. The polypeptide of claim 1, which is obtainable by bringing into expression a human or mouse gene in a host of animal, plant or microbe origin.

3. The polypeptide of claim 1, wherein said gene contains a part or whole of the nucleotide sequence of SEQ ID NO:1 or 2.

4. The polypeptide of claim 1, wherein said gene contains the nucleotide sequence of SEQ ID NO:3 or 4.

5. The polypeptide of claim 1, wherein said gene contains either nucleotide sequence of SEQ ID NOs:5 to 11.

6. The polypeptide of claim 1, which contains one or more amino acid sequences of SEQ ID NOs:12 to 19.

7. The polypeptide of claim 1, which contains a part or whole of the nucleotide sequence of SEQ ID NO:20 or 21.

8. The polypeptide of claim 1, which contains either amino acid sequence of SEQ ID NOs:22 to 25.

9. An agent for interleukin-18 receptor susceptive diseases, which contains as effective ingredient the polypeptide of claim 1.

10. The agent of claim 9, which contains as stabilizer a protein, saccharide and/or buffer.

11. An anti-autoimmune disease agent in accordance with claim 9.

12. An immunosuppressant in accordance with claim 9.

13. A DNA which encodes the polypeptide of claim 1.

14. The DNA of claim 13, which originates from human or mouse.

15. The DNA of claim 13, which contains a part or whole of either nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2 or their complementary sequence.

16. The DNA of claim 13, which contains either nucleotide sequence of SEQ ID NO:3, SEQ ID NO:4 or their complementary sequence.

17. The DNA of claim 13, which contains either nucleotide sequence of SEQ ID NO: 5, SEQ ID: 6, SEQ ID NO: 7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11 or their complementary sequence.

18. The DNA of claim 13, wherein, based on the degeneracy of genetic codes, one or more nucleotides are replaced with different nucleotides while conserving the amino acid sequence.

19. The DNA of claim 13, which is placed in an autonomously replicable vector.

20. The DNA of claim 13, which is placed in a host of animal, plant or microbe origin.

21. A process to prepare polypeptide, comprising bringing into expression a DNA which encodes the polypeptide of claim 1; and collecting the resultant polypeptide.

22. The process of claim 21, wherein the resultant polypeptide is collected through a step which includes salting out, dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatography, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis and/or isoelectric focusing gel electrophoresis.

23. The process of claim 21, wherein the resultant polypeptide is collected through a step which includes immunoaffinity chromatography using monoclonal antibody.

24. A agent to neutralize interleukin-18, which contains as effective ingredient the polypeptide of claim 1.

25. A method to neutralize interleukin-18, characterized by allowing the polypeptide of claim 1 to act on interleukin-18.

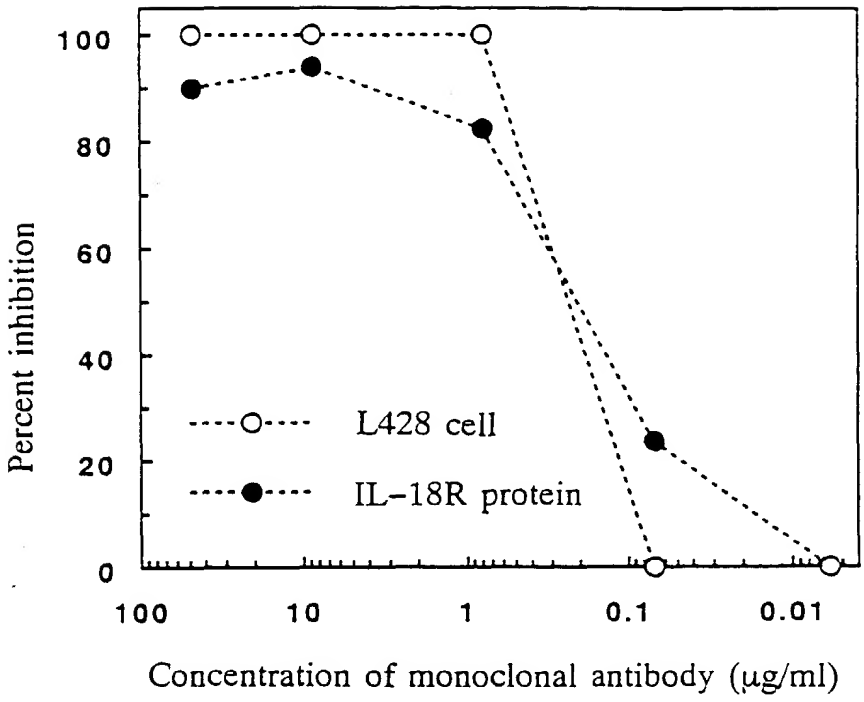
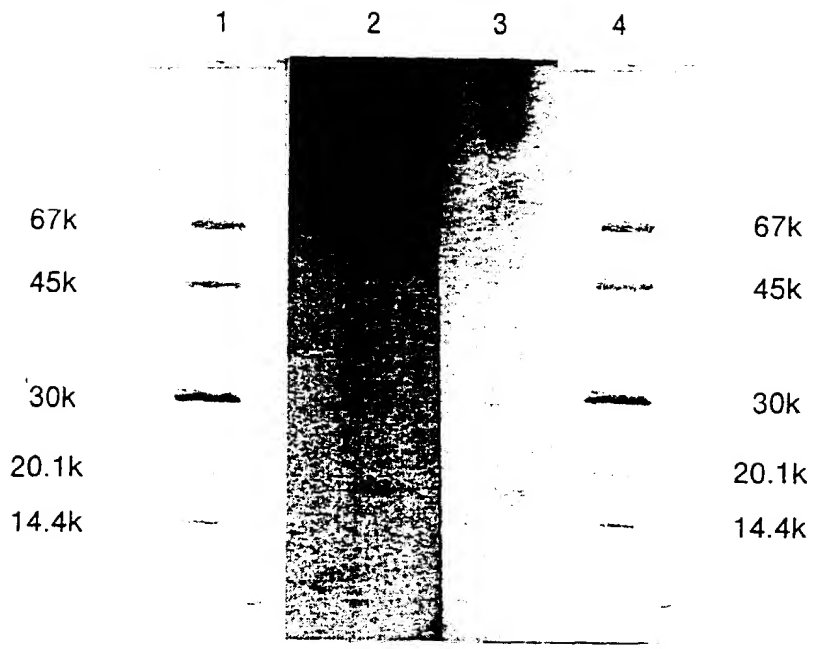

100
80
60
40
20
0
Percent inhibition
L428 cell
IL-18R protein
100
10
1
0.1
0.01
Concentration of monoclonal antibody (μg/ml)

FIG. 1



Lane 1 : Molecular weight markers
Lane 2 : Sample (with monoclonal antibody)
Lane 3 : Sample (without monoclonal antibody)
Lane 4 : Molecular weight markers

FIG. 2

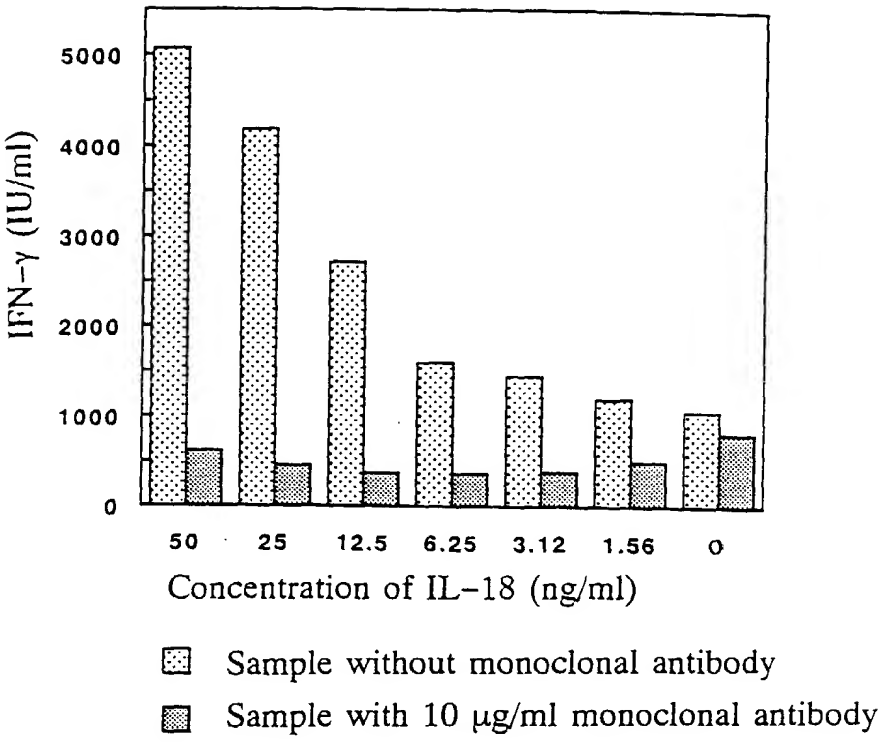

IFN-γ (IU/ml)
5000
4000
3000
2000
1000
0
50
25
12.5
6.25
3.12
1.56
0
Concentration of IL-18 (ng/ml)
Sample without monoclonal antibody
Sample with 10 μg/ml monoclonal antibody

FIG. 3

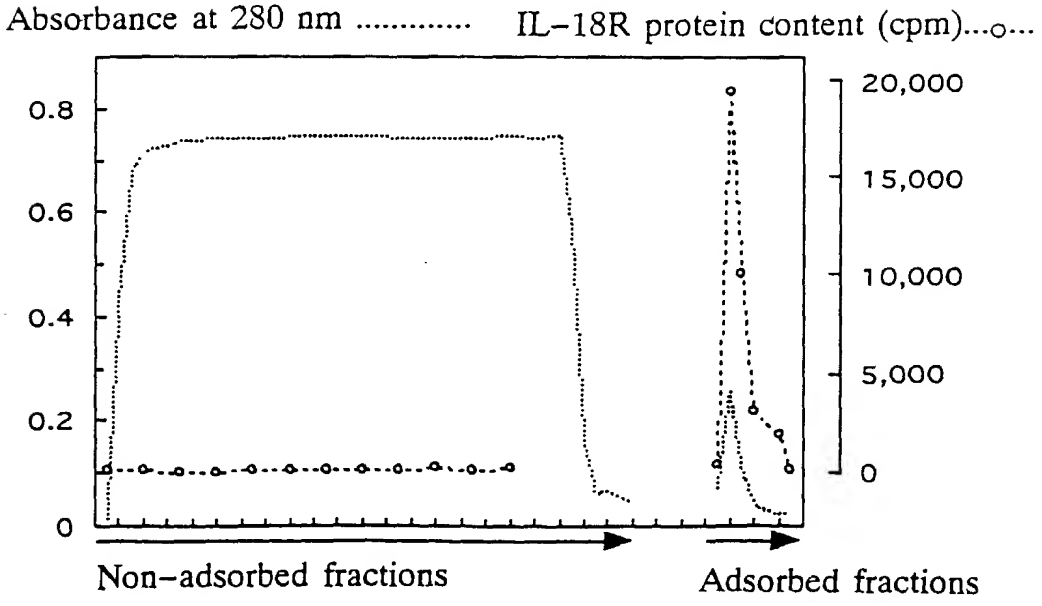

Absorbance at 280 nm .....
IL-18R protein content (cpm)...o...
0.8
0.6
0.4
0.2
0
20,000
15,000
10,000
5,000
0
Non-adsorbed fractions
Adsorbed fractions

FIG. 4

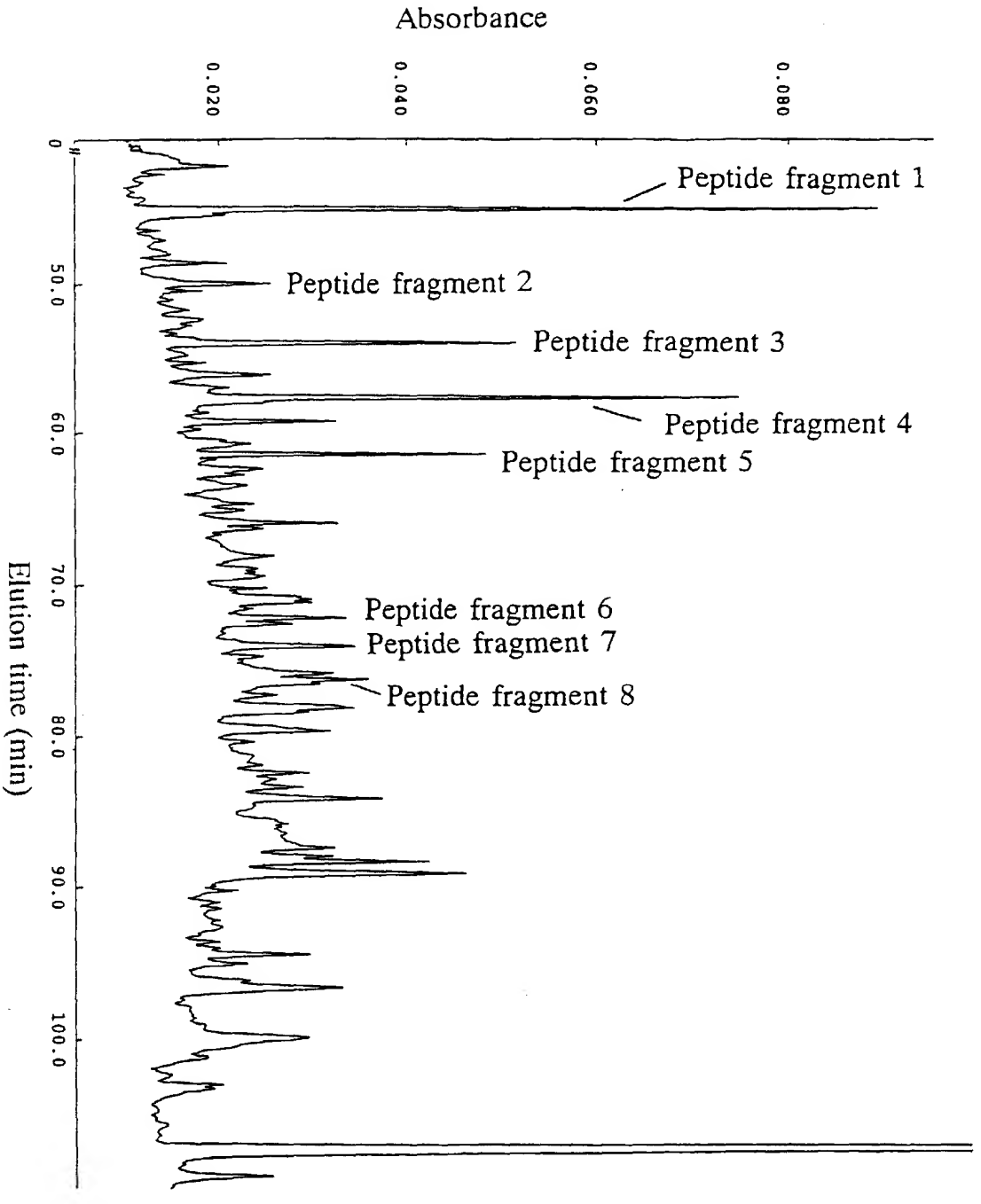

Absorbance
0.020
0.040
0.060
0.080
0
50.0
60.0
70.0
80.0
90.0
100.0
Elution time (min)
Peptide fragment 1
Peptide fragment 2
Peptide fragment 3
Peptide fragment 4
Peptide fragment 5
Peptide fragment 6
Peptide fragment 7
Peptide fragment 8

FIG. 5

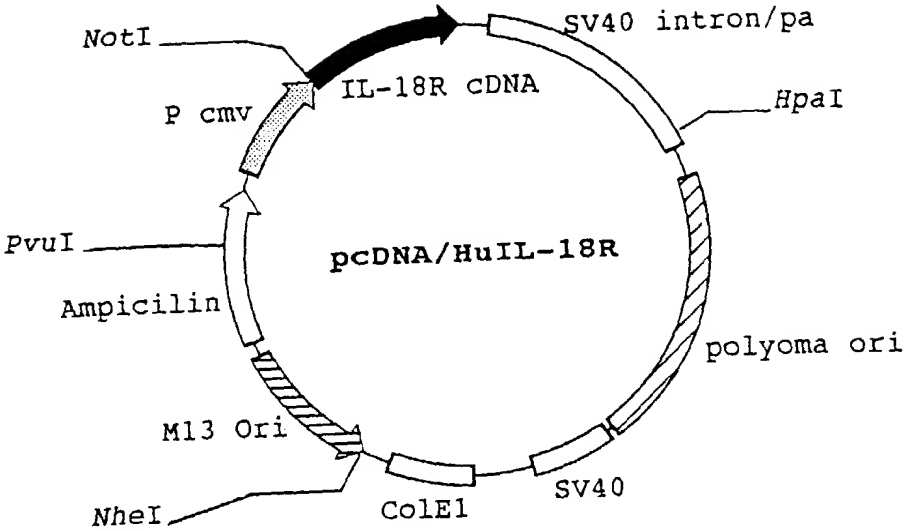

NotI
SV40 intron/pa
IL-18R cDNA
P cmv
HpaI
PvuI
pcDNA/HuIL-18R
Ampicilin
polyoma ori
M13 Ori
NheI
ColE1
SV40

FIG. 6

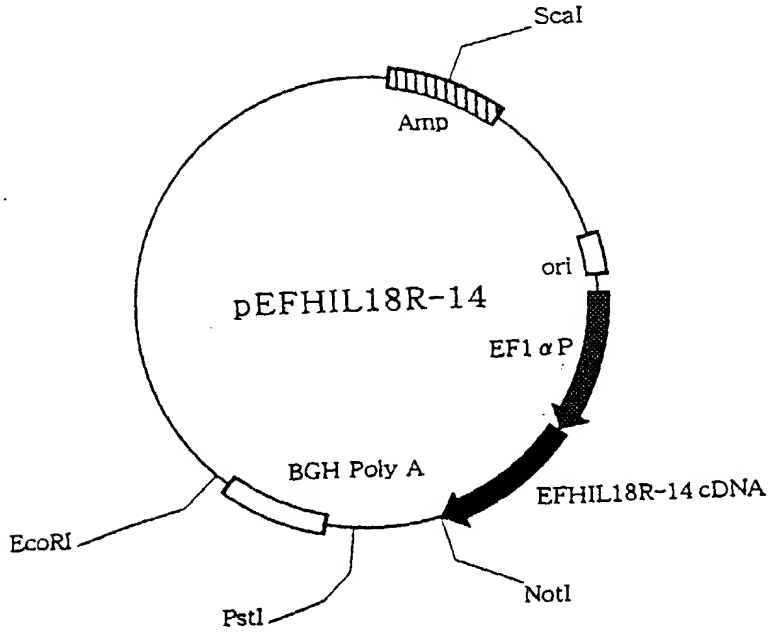

ScaI
Amp
ori
pEFHIL18R-14
EF1αP
BGH Poly A
EFHIL18R-14 cDNA
EcoRI
NotI
PstI

FIG. 7

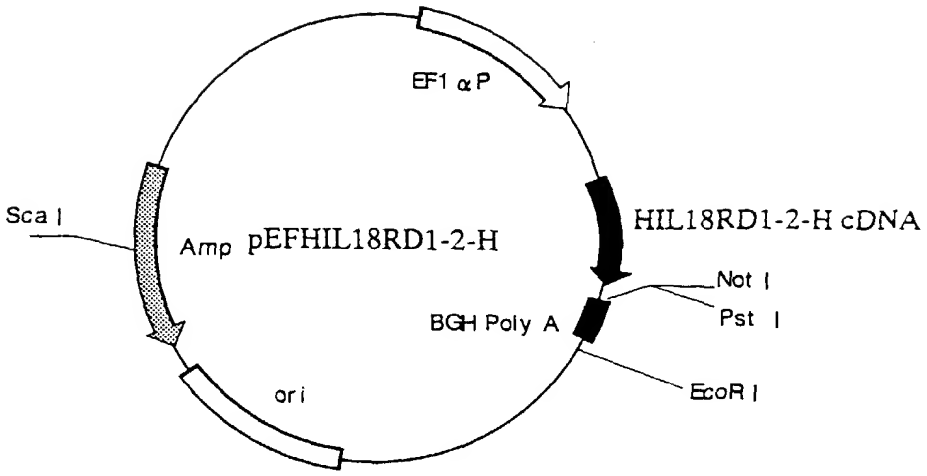

EF1 α P
Sca I
Amp pEFHIL18RD1-2-H
HIL18RD1-2-H cDNA
Not I
Pst I
BGH Poly A
ori
EcoR I

FIG. 8

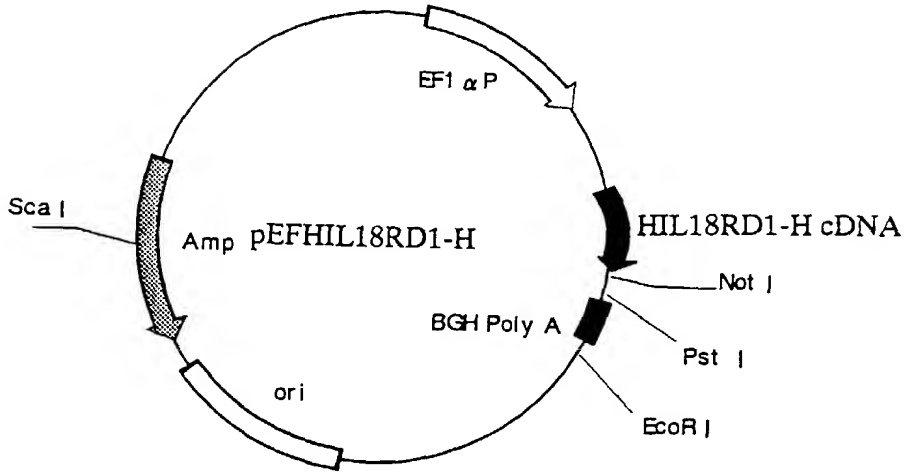

EF1 α P
Sca I
Amp pEFHIL18RD1-H
HIL18RD1-H cDNA
Not I
BGH Poly A
Pst I
ori
EcoR I

FIG. 9

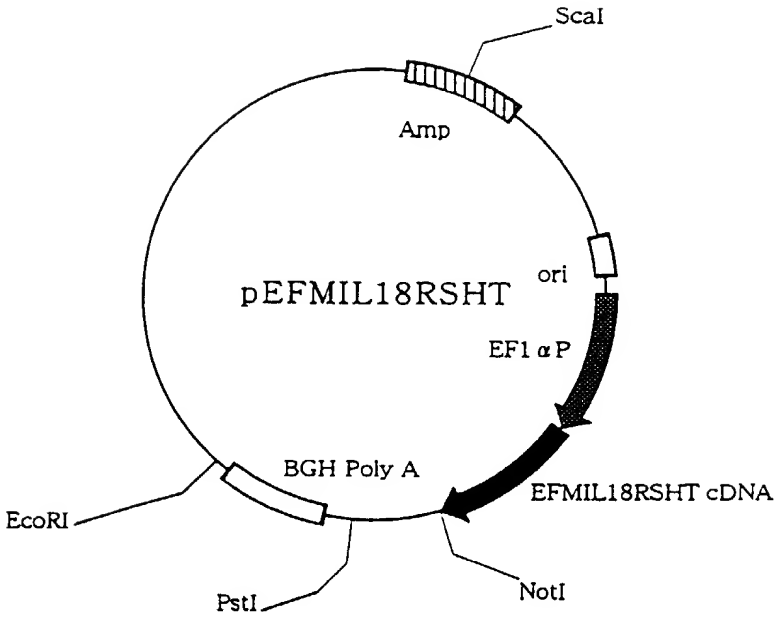

ScaI
Amp
pEFMIL18RSHT
ori
EF1αP
BGH Poly A
EFMIL18RSHT cDNA
EcoRI
PstI
NotI

FIG. 10

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 31 0517

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	PARNET, P. ET AL.: "IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologs T1/ST2 and IL-1R AcP" J. BIOL. CHEM., 271(8), 3967-70, 23 February 1996, XP002059933 * figure 1 * ---	1-11,27	C07K14/715 A61K38/17 A61K39/00
P,X	WO 97 31010 A (IMMUNEX CORP) 28 August 1997 * the whole document * -----	1-28	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	25 March 1998	Chakravarty, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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